University of Bath

PHD

Reperfusion induced arrhythmias in the isolated rat heart: The role of oxygen free radicals and the ionic environment of the heart.

Zakaria, Mohamed Naguib Mohamed

Award date:
1985

Awarding institution:
University of Bath

Link to publication

Alternative formats
If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

Copyright of this thesis rests with the author. Access is subject to the above licence, if given. If no licence is specified above, original content in this thesis is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC-ND 4.0) Licence (https://creativecommons.org/licenses/by-nc-nd/4.0/). Any third-party copyright material present remains the property of its respective owner(s) and is licensed under its existing terms.

Take down policy
If you consider content within Bath's Research Portal to be in breach of UK law, please contact: openaccess@bath.ac.uk with the details. Your claim will be investigated and, where appropriate, the item will be removed from public view as soon as possible.

Download date: 05. May. 2023

REPERFUSION INDUCED ARRHYTHMIAS IN THE ISOLATED RAT HEART
THE ROLE OF OXYGEN FREE RADICALS AND THE IONIC ENVIRONMENT OF THE HEART

Submitted by Mohamed Naguib Mohamed Zakaria

for the degree of Doctor of Philosophy

of the University of Bath

1985

COPYRIGHT

Attention is drawn to the fact that copyright of this thesis rests with its author. This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the prior written consent of the author.

This thesis may be made available for consultation within the University Library and may be photocopied or lent to other Libraries for the purposes of consultation.

SIGNED:

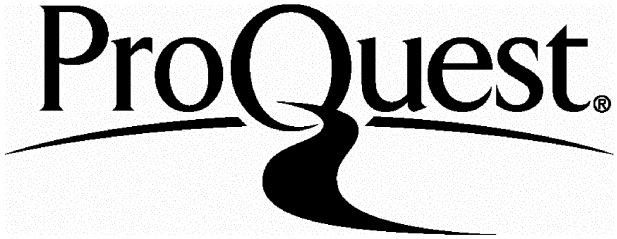
M. Naguib M. Zakaria

ProQuest Number: U362907

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.



ProQuest U362907

Published by ProQuest LLC(2015). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code.
Microform Edition © ProQuest LLC.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

"To the soul of my mother"

ACKNOWLEDGEMENTS

It is with a sense of deepest gratitude that I acknowledge the prodigious efforts of my supervisor, Dr. B. Woodward, for his day-to-day supervision, constant interest and enlightened cooperation.

I am grateful to the University of Zagazig for the scholarship awarded to me.

I would like to express my gratitude to my wife for her being patient and for her encouragement.

Finally, I would like to thank Mrs. Judy Harbutt for her efficient typing.

SUMMARY

Reperfusion of the isolated rat heart following 10 min of coronary artery ligation under constant flow conditions results in the development of arrhythmias - premature ventricular contractions (PVCs), ventricular tachycardia (VT) and ventricular fibrillation (VF).

Increasing concentrations of magnesium (0 - 4.8 mM) and/or potassium (2.5 - 10.0 mM) attenuate, while clacium (0.6 - 2.4 mM) exacerbates these arrhythmias. The protective effects of magnesium and potassium were additive. Magnesium reduced heart rate, perfusion pressure and developed tension. Potassium reduced perfusion pressure and increased developed tension. These haemodynamic effects contributed to the antiarrhythmic action of magnesium but did not completely account for its antiarrhythmic action. Calcium increased developed tension and heart rate and reduced perfusion pressure. Post-ligation administration of magnesium and potassium also protected against reperfusion arrhythmias. These results demonstrate that reperfusion arrhythmias are significantly affected by the ionic environments of the heart.

Superoxide dismutase (5 - 20 μml^{-1}), glutathione (10^{-5} - 10^{-3}M), ascorbic acid (10^{-4} - 5 x 10^{-4} M) and histidine (5 x 10^{-3} M) when given before coronary artery ligation attenuated the development of reperfusion arrhythmias. Mannitol (2 x 10^{-2} M) and catalase (100 - 300 μml^{-1}) did not have any significant effect on reperfusion arrhythmias when given alone but they did potentiate the effect

of superoxide dismutase. Glutathione and a combination of superoxide dismutase, catalase and mannitol also reduced the incidence of reperfusion induced ventricular fibrillation when given just before reperfusion. Ferrous ion exacerbated the severity of reperfusion arrhythmias. Mannitol (2×10^{-2}M), catalase (100 μml^{-1}) and histidine (5×10^{-3} M) when given before coronary ligation or just before reperfusion prevented the effect of ferrous ion while superoxide dismutase did not, indicating that the presence of ferrous ion is important for the production of hydroxyl radicals. Pretreatment with 6-OHDA attenuated the incidence of reperfusion arrhythmias but pre-ligation administration of allopurinol had no effect on reperfusion arrhythmias. By perfusing hearts with ferricytochrome C it was possible to show an increased reduction of ferricytochrome C during the first minute of reperfusion which could be prevented by the addition of superoxide dismutase and 6-OHDA treatment. These results provide evidence that oxygen free radicals are produced and may be important in the genesis of reperfusion induced arrhythmias in the isolated rat heart.

Adenosine (10^{-6} M), verapamil (10^{-8} - 10^{-7} M), ZK 36374 (10^{-10} - 10^{-9} M) and sodium nitroprusside (10^{-6} M) attenuate the incidence of reperfusion arrhythmias which may be via a coronary steal effect. Agents which affect arachidonic acid metabolism yielded conflicting results which may reflect nonspecific mechanisms other than inhibition of arachidonic acid metabolism.

Glutathione and a mixture of superoxide dismutase, catalase and mannitol when given before coronary ligation and just before

reperfusion reduced the increase in ^{86}rubidium efflux rate constant shown on reperfusion. The effect of glutathione on ^{86}rubidium efflux may be at least in part due to its vasodilator effect. Superoxide generation by xanthine/xanthine oxidase system increased the rate of efflux of ^{86}rubidium. A mixture of superoxide dismutase, catalase and mannitol also reduced the transient increase in the rate of release of ^{3}H-noradrenaline shown to be produced on reperfusion after 10 min of ischaemia in the isolated rat heart.

CONTENTS

CHAPTER 1

INTRODUCTION

A. Reperfusion induced cardiac arrhythmias

1.1: Definition and clinical relevance

Reperfusion induced cardiac arrhythmias are the disturbances of heart rhythm that arise as a consequence of a total or partial restoration of flow to tissue which has been previously globally or regionally ischaemic (Manning and Hearse, 1984). This type of arrhythmia is often severe and is seen in most species studied (Sheridan *et al.*, 1980; Bergey *et al.*, 1982; Woodward and Zakaria, 1983) and in man (Goldberg *et al.*, 1983). Usually these arrhythmias are sudden in onset and deteriorate to ventricular fibrillation within 5-30 seconds (Kaplinsky *et al.*, 1981). The immediate causes of these arrhythmias are poorly understood (Murdock *et al.*, 1980; Kaplinsky *et al.*, 1981; Woodward and Zakaria, 1985) and are the subject of this thesis.

Although the advent of coronary bypass surgery has made reperfusion induced arrhythmias relevant to the clinical setting (Levites *et al.*, 1975), the applicability of reperfusion to human arrhythmias including sudden cardiac death is controversial (Corr and Witkowski, 1984). In a large percentage of patients autopsied after sudden cardiac death, there is an absence of complete coronary occlusion, suggesting that the lethal event may have followed an ischaemic interval after subsequent reperfusion via one or more of the following mechanisms: (1) Platelet aggregation with thrombus formation and subsequent spontaneous lysis (or recanalization by the use of thrombolytic therapy in patients during acute myocardial infarction). (2) Spasm followed by relaxation

of a normal or partially obstructed artery. (3) Increase in collateral flow to an ischaemic region (Corr and Witkowski, 1984). Moreover, Goldberg *et al.* (1983) have reported that during intracoronary thrombolysis in patients, the presence of ventricular arrhythmias is an indicator of the success of recanalization. Parmley and Chatterjee (1984) in a recent review have pointed out that spontaneous thrombolysis frequently occurs and is usually too late to salvage ischaemic myocardium, but intravenous thrombolytic agents can produce relatively rapid thrombolysis, reestablish flow and in some cases salvage ischaemic myocardium.

1.2: Electrophysiological derangements associated with reperfusion

Studies of the effects of ischaemic blood obtained from a local coronary vein (on release of a coronary artery occlusion) on transmembrane potentials of muscle strips taken from the same heart (Downar *et al.*, 1977a) and intracellular electrode recordings from the subepicardium of the ischaemic zone (Downar *et al.*, 1977b) have revealed that ischaemia results in a gradual, heterogeneous, progressive decrease in action potential duration, resting membrane potential and maximum rate of rise of voltage of phase 0 (V max). These deteriorations occur over a period of minutes and are most pronounced in the centre of the ischaemic zone. Moreover, Ramanathan *et al.* (1977) reported a significant shortening of refractoriness in the ischaemic zone which results in an inhomogeneity of ventricular refractoriness between the ischaemic and non-ischaemic zones, and a unidirectional delay of conduction from ischaemic to non-ischaemic zones predisposing to reentry mechanism. Similarly,

Murdock *et al.* (1980) have concluded that ischaemia results in a gradual conduction slowing establishing reentrant pathways followed by a further conduction suppression so that reentrant pathways become blocked.

Abrupt reperfusion of the ischaemic region was reported to result in a heterogeneous recovery of electrical activity (Downar *et al.*, 1977a), either further shortening (Naimi *et al.*, 1977) or suddenly prolonging refractory periods (Levites *et al.*, 1975) resulting in increased dispersion of refractoriness between ischaemic and non-ischaemic myocardium (Corr and Witkowski, 1984) and rapid but brief decrease in the ventricular fibrillation threshold (Axelrod *et al.*, 1975; Corbalan *et al.*, 1976). Murdock *et al.*, (1980)have concluded that reperfusion of the electrically unresponsive tissue results in a nonhomogeneous improvement in conduction that transiently predispose to reenetry mechanism. Downar *et al.* (1977a) also related the extent of heterogeneity of recovery times to be a key determinant of ventricular fibrillation threshold.

As ventricular arrhythmias are generally believed to be due to either re-entry or enhanced automaticity, the dispersion of refractoriness between ischaemic and nonischaemic myocardium (Levites *et al.*, 1975; Kaplanisky *et al.*,1981) as well as nonhomogenous recovery of conduction (Murdock *et al.*, 1980) that follows reperfusion were reported to be a possible cause of re-entry mechanism. On the other hand, experiments on the role of enhanced automaticity during reperfusion yielded conflicting results (Murdock *et al.*, 1980).

Increases in ventricular automaticity have been reported after abrupt reperfusion of ischaemic region (Kaplinsky *et al.*, 1981), however, others have reported that idioventricular rate either remained unchanged(Rama nathan *et al.*, 1977; Murdock *et al.*, 1980)or decreased with reperfusion (Levites *et al.*, 1975). In humans undergoing intra-coronary thrombolysis, accelerated idioventricular rhythms are common (Corr and Witkowski, 1984).

It would appear from the above that the precise electro-physiologic derangements responsible for reperfusion induced dysrhythmias are not clear (Murdock *et al.*, 1980), but may be due to heterogeneous electrical recovery leading to re-entry mechanism and possibly, enhanced ventricular automaticity and this might provide the necessary electrophysiological background for the genesis of reperfusion induced arrhythmias.

1.3: Biochemical factors associated with myocardial ischaemia and reperfusion

Acute myocardial ischaemia has been reported to induce the following biochemical changes: (1) Rapid depletion of nucleotide pools (ATP, GTP and CP) and increase in myocardial content of adenine nucleotide catabolites (Cyclic AMP, adenosine, inosine and hypoxanthine) (Downar *et al.*, 1977a; Opie *et al* ., 1979: Swain *et al.*, 1984) which may directly affect maintenance of ionic gradients and ionic conductance across the sarcolemma leading to, (2) an increase in extracellular potassium and probably intracellular calcium (Downar *et al.*, 1977a; Opie *et al.*, 1979) and (3) release of intramyocardial

catecholamines (Abrahamsson *et al.*, 1983). (4) Hypoxia and increased pCO_2 leading to switching off of anaerobic glycolysis and an elevation of extracellular and tissue lactate levels, acidosis and hypoglycaemia (Downar *et al.*, 1977a; Opie *et al* ., 1979). (5) Accumulation of lysophosphoglycerides (Sobel *et al.*, 1978).

The exact biochemical abnormalities contributing to abnormal function and electrophysiological derangement after myocardial ischaemia and reperfusion are not known (Kloner *et al* ., 1983). Although Downar *et al.* (1977a) have demonstrated that the combined effects of acidosis, hypoxia, high lactate levels, hyperkalaemia, low ATP levels and hypoglycaemia could not produce the electrophysiological changes produced by blood collected from local coronary vein on release of coronary artery occlusion and they attributed these electrophysiological changes to unidentified substances released from ischaemic tissue, other investigators reported that ischaemic blood obtained from pigs after 15 minutes of occlusion has adverse electrophysiological effects causing shortening of action potential duration, loss of resting membrane potential and reduction in upstroke velocity when introduced into the bath of normal muscle strips (Opie *et al.*, 1979; Saman and Opie, 1984). Similar electrophysiological derangements have been reported to be produced by hyperkalaemia (Opie *et al.*, 1979). Furthermore, acidosis and a high pCO_2 have been reported to augment the electrophysiological derangements induced by accumulation of lysophosphoglycerides (Corr *et al.*, 1982) and dibutyryl cyclic AMP to lower ventricular fibrillation threshold in the isolated rat heart (Opie *et al.*, 1979).

Reperfusion of ischaemic myocardium has been shown to result in slow recovery of nucleotide pools (Swain *et al.*, 1984). The rapid and heterogeneous reversal of the elevated extracellular potassium to control levels may predispose to reperfusion induced arrhythmias (Corr and Witkowski, 1984), the wash out of products liberated from ischaemic and necrotic cells may also contribute to genesis of reperfusion arrhythmias (Axelrod *et al.*, 1975; Opie *et al.*, 1979), these possibilities are supported by the rapid onset of electrical instability within seconds after reperfusion (Corr and Witkowski, 1984).

The contradictory reports mentioned above show that the precise biochemical factors leading to the development of arrhythmias are not understood, indeed the cause may not even be due to biochemical factors.

B. Factors influencing reperfusion induced arrhythmias

1.4: Duration and severity of the preceding period of ischaemia

The duration of the preceding ischaemic period as being an important factor in determining the severity of reperfusion induced arrhythmias is well documented (Balke *et al* ., 1981; Crome *et al.*, 1983). From studies with anaesthetized dog, duration of preceding ischaemia has been reported to directly influence the incidence of dysrhythmias associated with reperfusion (Balke *et al.*, 1981). In both, the isolated working rat heart (Crome *et al.*, 1983) and the

anaesthetized rat (Manning and Hearse, 1984), the incidence of reperfusion induced ventricular fibrillation has been shown to be increased with increasing periods of ischaemia until it reaches the maximum, then, as the duration of ischaemia increases there is a progressive decline in the incidence of reperfusion induced arrhythmias suggesting that the highest incidence of reperfusion induced ventricular fibrillation occurs after a time during which some cells may begin to demonstrate irreversible damage.

The severity of the incidence of reperfusion induced arrhythmias might also be expected to be dependent upon the severity of the preceding ischaemia. Kaplinsky *et al.*, (1981) have pointed out a positive correlation between the incidence of arrhythmias occurring during ischaemia and those occurring during reperfusion. Therefore, reducing the severity of ischaemia may act to slow injury, delay its onset and irreversibility and thus displace the maximal incidence of reperfusion arrhythmias to a later time (Manning and Hearse, 1984). But complete global ischaemia is not associated with reperfusion arrhythmias.

It was of interest to find out the optimal period of ischaemia which predisposes to the highest incidence of reperfusion induced arrhythmias in the isolated rat heart, to guide extension of the present study.

In the initial experiments reported in this thesis, the time course of reperfusion arrhythmias were studied in the isolated rat heart.

1.5: Animal Species

It is known that differences among species regarding heart size, heart rate, collateral circulation, etc. may affect both arrhythmogenesis and drug effects in various arrhythmia models (Bergey *et al.*, 1982). Therefore, a critical heart size has been reported to be necessary to sustain ventricular fibrillation as small hearts are prone to spontaneous defibrillation particularly if a re-entrant or circus mechanism is the cause of arrhythmias, while in large hearts where reentrant pathways may be relatively longer than in small hearts a tendency to spontaneous defibrillation is not seen (Bergey and Beil, 1983). The canine myocardium for example has been reported to have an extensive although variable degree of collateral blood supply which may lead to a low incidence of arrhythmias and contradictory results in this species (Balke *et al.*, 1981; Bergey *et al.*, 1982).

1.6: Are reperfusion arrhythmias a flow or Oxygen-dependent phenomenon?

The process of reperfusion not only restores nutrients and flow to ischaemic myocardium but also reoxygenates hypoxic tissue, therefore, either component of the process may contribute to the resulting arrhythmias. What has been reported by Carbonin *et al.* (1981) is that in globally ischaemic hearts, anoxic perfusate had a protective effect against reperfusion induced arrhythmias. This provides some evidence for the role of reoxygenation in arrhythmogenesis. Hess and Manson (1984) have pointed out that reoxygenation of hypoxic hearts results in significant damage of both phospholipid membrane

structures and myofibrils. On the contrary, it has been reported that reperfusion of ischaemic myocardium with venous blood or anoxic saline does not appear to alter the incidence of reperfusion induced ventricular fibrillation (Petropoulos and Meijne, 1964), ischaemic hearts being more vulnerable to ventricular fibrillation than hearts made hypoxic without flow reduction (Corr and Witkowski, 1983). The question of which component of reperfusion is the most important contributory factor in reperfusion arrhythmogenesis is therefore not clear.

1.7: How sudden is reperfusion of the ischaemic myocardium

Ventricular fibrillation associated with reperfusion has been reported to be abolished or decreased by decreasing the rate of reperfusion (Corbalan *et al.*, 1976). Similarly, Petropoulos and Meijne (1964) had shown that gradual rather than rapid reperfusion has been shown to attenuate significantly the associated ventricular arrhythmias suggesting that rapid changes across the reperfusion region may be a contributing factor.

The effect of changing reperfusion rate has been examined in this thesis.

1.8: Potassium status

Potassium plays a major determinative role in maintaining the electrophysiological stability of cardiac function (Schwartz, 1978). The normal extracellular potassium concentration is 3.5 to 5.5 mM which represents about 1.5 - 2.0% of the total body potassium, while the intracellular concentration averages are about 150 to 160 mM

(Schwartz, 1978; Nayler, 1981). This gradient is maintained by virtue of the active Na^+ - K^+ pump mechanism.

Alkalosis, glucose, insulin (by shifting potassium into cells without net change in total body potassium) and diuretics (by increasing kaluresis) may lower the extracellular potassium concentration, while acidosis (by shifting potassium out of cells without net change in total body potassium) and tissue trauma may elevate the extracellular potassium concentration (Schwartz, 1978).

Potassium carries the delayed repolarizing currents that are thought to comprise of three components Ixl and Ix2 responsible for the Purkinje fibre plateau and IK_2 for spontaneous diastolic depolarization (Gettes, 1981). Changes in extracellular K^+ affect the electrophysiology of cardiac cells. The resting membrane potential, depolarizing currents, action potential plateau duration and slopes of rapid repolarization and spontaneous diastolic depolarization are all influenced by extracellular K^+ (Gettes, 1981; Vassalle, 1981).

Schwartz (1978) has reported that hypokalaemia causes an increase in resting membrane potential, increases phase 4 of depolarization (which represents the ability of the cell to depolarize by itself) and decreases the threshold potential. Enhanced automaticity thought to be caused by decreased potassium conductance may occur in the sinus node and in other tissues of the heart particularly the AV junction leading to junctional tachycardias and arrhythmias of multifocal origin. Hypokalaemia may also depress conduction,

thus hypokalaemia arrhythmias may also be caused by enhancement of reentry. On the contrary, hyperkalaemia causes a reduction in the resting membrane potential which leads to an increased excitability and may produce enhanced automaticity. Further increases in extracellular K^+ concentration decrease excitability until it becomes entirely lost. Hyperkalaemia also decreases the rate of rise of phase 4 of depolarization which would reduce automaticity. This decrease in resting membrane potential decreases the upstroke velocity of phase 0 of the transmembrane action potential, resulting in reduced conduction velocity (Schwartz, 1978). In the previous settings, it is possible to have any of a number of combinations of changes, for example hyperkalaemia may increase, decrease or have no effect on automacitiy (Schwartz, 1978).

Links between K^+ and arrhythmias were first described in 1954 by Harris and his co-workers when extracellular K^+ accumulation was reported during myocardial ischaemia. Since then many reports have confirmed the antiarrhythmic effect of K^+salts (Cranfield and Wit, 1979; Opie *et al.*, 1979; Andersson, 1980; Nayler, 1981; Woodward and Zakaria, 1983). In isolated rat hearts, the perfusate K^+ concentration was found to be a major determinant of ventricular fibrillation in both ligation and reperfusion arrhythmias (Lubbe *et al.*, 1978).

1.9: Magnesium status

Magnesium is the second most common intracellular cation next to potassium. It plays a vital role in many cell processes such as

activation of the membrane bound ATPase enzyme responsible for the Na^+ - K^+ pump mechanism essential for restoring cell K^+ and preserving the integrity of the cell membrane (Iseri *et al.*, 1975; Whang *et al.*, 1980). Magnesium is also essential for the oxidative phosphorylation processes necessary for the replenishment of ATP essential for contractility of the cardiac muscle (Johnson *et al.*, 1979).

The normal extracellular magnesium concentration is about 0.9 mM (Nayler, 1981a), half of which is bound to protein (Shine, 1979), while the normal intracellular magnesium concentration in cardiac muscle is about 15 mM (Nayler, 1981a), 12% of which has been estimated to be in the mitochondria, 2 - 3% in the myofibrils and a large proportion is complexed with ATP, ADP, AMP and enzyme-coenzyme complexes (Shine, 1979).

Hypomagnesaemia may exist due to intake of potent diuretics (e.g. frusemide), hyperaldosteronism, acute or chronic alcoholism, acidosis, hypoparathyroidism and glucosuria which promote urinary excretion of magnesium (Iseri *et al.*, 1975), while impaired excretion due to renal failure and excessive intake of Mg^{2+} salts used as laxatives may cause hypermagnesaemia.

Alterations of Mg^{2+} concentration may have little effect upon the cardiac action potential. High serum Mg^{2+} has been reported to cause decreased AV conduction, intraventricular conductance and suppression of sinus node function leading to bradycardia (Shine, 1979) and prolonged action potential duration (Johnson *et al.*, 1979).

Magnesium deficiency was shown to affect the distribution of potassium (Iseri *et al.*, 1975; Johnson *et al.*, 1979; Whang *et al.*, 1980; Nayler,1981a) and calcium, (Shine and Douglas, 1975; Shine, 1979; Dyckner and Wester ,1980; Altura *et al.*, 1981) across the cell membrane and is frequently associated with cardiac arrhythmias (Iseri *et al.*, 1975; Shine, 1979; Dyckner and Wester, 1980; Nayler, 1981a). Mg^{2+} may be as important as potassium in coronary heart disease (Johnson *et al.*, 1979) and shows a clinical effectiveness when used as antiarrhythmic medication (Iseri *et al.*,, 1975; Dyckner and Wester, 1980).

The decreased Mg^{2+} and K^{+} concentrations in the myocardial tissue in men dying suddenly from myocardial infarction and the necessity to optimise tissue concentrations of both Mg^{2+} and K^{+} for the maintenance of normal cardiac function (Johnson *et al.*, 1979) suggested that it was worthwhile to study the effect of Mg^{2+}/K^{+} ratios on the incidence of reperfusion induced arrhythmias to confirm and extend the reports mentioned above. The results of these experiments are presented in Chapter 4.

1.10: Calcium status

Calcium ions carry the slow inward current responsible for the depolarization phase of action potential in the sinoatrial node, atrioventricular node and Purkinje fibres when the fast sodium current is blocked (Andersson, 1980) or during the plateau phase (Vassalle, 1981). A second type of Ca^{2+} dependent ionic current that occurs is the Na^{+}/Ca^{2+} exchange mechanism, this may mediate arrhythmias

when membrane bound ATPase is inhibited (Nayler, 1981a: Clusin *et al.*, 1982).

The normal extracellular Ca^{2+} concentration is 2.0 - 2.4 mM, about half of which is bound in nonionized complexes, and the normal intracellular Ca^{2+} concentration is about 10^{-7} - 10^{-5} M (Nayler, 1981a).

Hypocalcaemia may be caused by impaired absorption of Ca^{2+} (due to avitaminosis), calcitonin, hypoparathyroidism (due to increased deposition of Ca^{2+} in bones), excessive loss (as during lactation), alkalosis and corticosteroids (by increasing the rate of Ca^{2+} excretion in urine). On the other hand, a high dietary load of Ca^{2+}, acidosis, vitamin D and hyperparathyroidism may cause hypercalcaemia.

High extracellular Ca^{2+} concentrations have been reported to shorten the action potential plateau, slightly increase the rate of diastolic depolarization (which may be carried in part by the slow inward current) and prolong atrioventricular conduction, while low extracellular Ca^{2+} concentration prolong action potential duration (Fish, 1973; Gettes, 1981) and suppress automaticity (Andersson, 1980). Furthermore, high levels of intracellular free calcium might cause accumulation of Ca^{2+} in the gap junction leading to increased junctional resistance and decreased conduction velocity (De Mello, 1982).

Fig. 1 (Nayler, 1981b) shows the possible sequence of events induced by ischaemia and reperfusion, but there is no evidence for

raised cytosolic Ca^{2+} during the first 30 minutes of ischaemia and this may be due to absence of very sensitive and efficient methods or instruments for measuring intracellular Ca^{2+} concentration which may be elevated but not detected.

However, calcium ions may be an important factor in mediating the adverse effects of cardiac ischaemia (Opie *et al.*, 1979; Clusin *et al.*, 1982) and reperfusion (Nayler, 1980). During reperfusion, tissue Ca^{2+} was reported to increase (Nayler, 1981a) and contribute to the precipitation of ventricular fibrillation (Clusin *et al.*, 1982).

Although the slow current may play a critical role in inducing arrhythmias (Opie *et al.*, 1979), studies with the drugs which block the calcium current have provided conflicting data as to their efficacy in preventing reperfusion induced arrhythmias. Verapamil has been shown to be effective in preventing reperfusion arrhythmias in some studies (Winslow *et al* ., 1983; Bergey *et al.*, 1984) but not in others (Kane *et al.*, 1984).

1.11: Free radicals

Free radicals may be defined as atoms or molecules that have an odd number of electrons in their outer orbital (Frank, 1983; Halliwell and Gutteridge, 1984; Hertz and Cloarec, 1984). They have been reported to be produced during myocardial ischaemia and reperfusion and to contribute to reperfusion induced tissue injury and arrhythmias (Meerson *et al.*, 1982; Hess and Manson, 1984; Otani *et al.*,, 1984; McCord, 1985; Van der Vusse and Reneman, 1985; Woodward

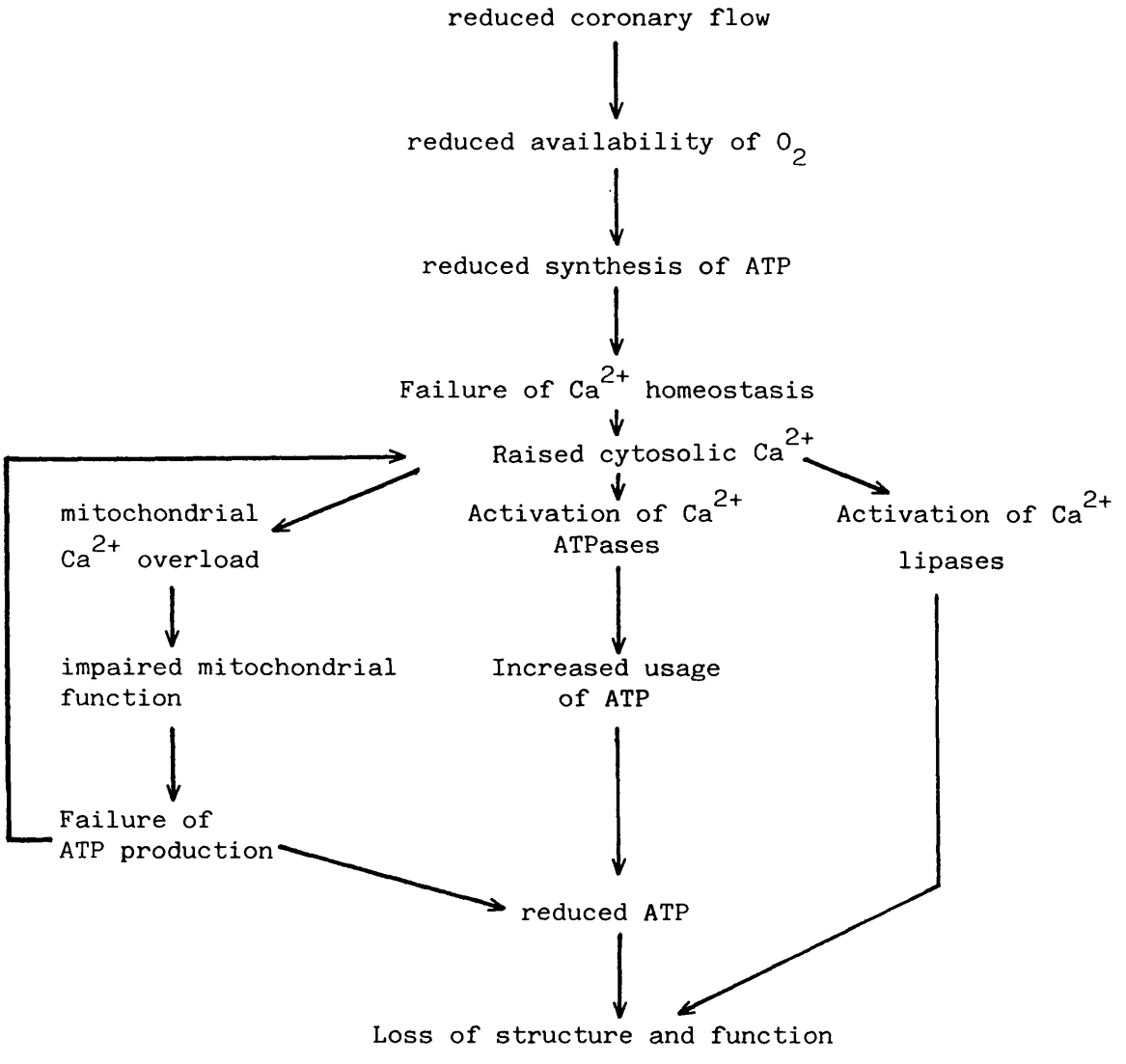


Fig. (1). Schematic representation of the possible sequence of events induced by myocardial ischaemia.

and Zakaria, 1985).

Oxygen reactive species including superoxide radicals ($O^{\cdot}_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\dot{O}H$) may be generated by a number of cellular reactions (Burton *et al.*, 1984). Fig. (2) shows the sequence of events which may lead to free radical production during myocardial ischaemia and reperfusion. Due to ischaemia or hypoxia, oxygen utilization may be switched from the oxidase pathway (which involves complete four electrons reduction of oxygen to water) to the oxygenase pathway (which involves step-wise reduction of oxygen by accepting one, two or three electrons) leading to the formation of the superoxide anion, hydrogen peroxide and the hydroxyl radical. Furthermore, on reperfusion, an excessive supply of oxygen is provided to the reduced metabolites accumulated in the ischaemic myocardium, this may lead to free radical production (Meerson *et al.*, 1982). Another source of superoxide anion production may be due to the autoxidation of catecholamines (Burton *et al.*, 1984) which have been reported to be released during reperfusion of the ischaemic myocardium (Rochette *et al.*, 1980). Moreover, free radicals have been reported to be produced during ischaemia (Van der Vusse and Reneman, 1985) as a result of arachidonic acid metabolism via both the lipo-oxygenase (Kontos *et al.*, 1984) and the cyclo-oxygenase pathways during the conversion of prostaglandin G_2 to the H_2 derivative (Rowe *et al.*, 1983; Hertz and Cloarc, 1984). In addition, xanthine oxidase has been reported also to be a major source of superoxide anion (Burton *et al.*, 1984). As a result of ischaemia, xanthine dehydrogenase (NAD^+ reducing enzyme) may convert to xanthine oxidase (superoxide producing enzyme) and this in the presence of high concentrations of adenosine, inosine and

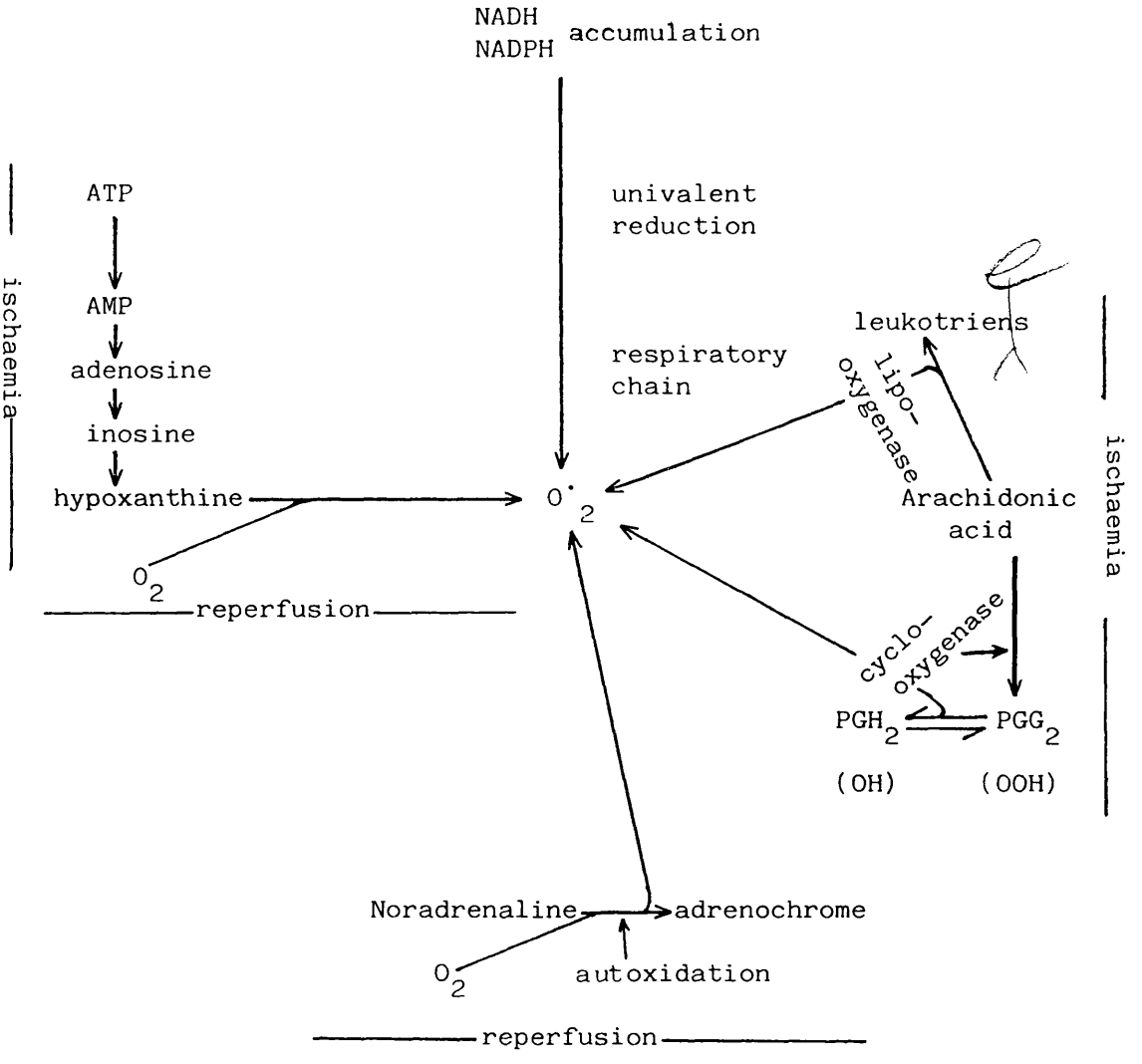


Fig. (2) The sequence of events which may lead to free radical generation during myocardial ischaemia and reperfusion

hypoxanthine which exist as a result of increased ATP degradation and decreased production, plus molecular oxygen supplied during reperfusion of the ischaemic tissue will result in superoxide anion production (McCord, 1985; Van der Vusse and Reneman, 1985).

Superoxide radicals can produce hydrogen peroxide according to the dismutation reaction (Frank, 1983; Rowe *et al.*, 1983; Hess and Manson, 1984):

$$O_2^{\cdot} + O_2^{\cdot} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (1)$$

This reaction can proceed spontaneously or it can be catalyzed by superoxide dismutase. Hydrogen peroxide can be converted by catalase to water, or interact with superoxide radicals according to the Haber-Weiss reaction under the catalytic effect of trace amounts of transition metals to generate the hydroxyl radical (Gutteridge, 1982; Rowe *et al.*, 1983; Halliwell and Gutteridge, 1984; Hess and Manson, 1984):

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2 \qquad (2)$$

$$H_2O_2 + O_2^{\cdot -} \xrightarrow{transition\ metal} O_2 + \dot{O}H + OH^- \qquad (3)$$

Haber Weiss reaction

Halliwell and Gutteridge, (1984) have reported that a simple mixture of hydrogen peroxide and ferrous salt can produce hydroxyl radicals via the Fenton reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + \dot{O}H + OH^- \qquad (4)$$

Moreover, they have pointed out that reduced ion chelates in the presence of molecular oxygen may generate superoxide radical via the formation of the perferryl complex:

$$Fe^{2+}(aq) + O_2 \rightleftharpoons Fe^{2+} - O_2 \rightleftharpoons Fe^{3+} - \dot{O}_2 \rightleftharpoons Fe^{3+} + \dot{O}_2$$

Under normal conditions, mitochondria generate a steady flux of superoxide radicals (Van der Vusse and Reneman, 1985). This flux is controlled by the intracellular scavenging defense mechanisms including superoxide dismutase, catalase, the GSH peroxidase-GSSG reductase cycle, α-tocopherol, ascorbic acid and reduced glutathione (Trush *et al.*, 1982; Frank, 1983). It is only when there is a decreased function of these protective mechanisms as in ischaemia (Van der Vusse and Reneman, 1985) or when there is excessive production of free radicals, that free radicals accumulate and exert their damaging effect (Meerson *et al.*,1982).

Free radicals have been reported to induce depolymerization of hyaluronic acid in synovial fluid, degradation of collagen, inactivation of enzymes (Brawn and Fridovich, 1980), damage to sarcoplasmic reticulum (Hess *et al.*, 1981; Jolly *et al.*, 1984) and peroxidation of lipids in the cell membrane (Meerson *et al.*, 1982) which will alter the ionic permeability of the sarcolemma. Free radicals, may also affect intracellular calcium sequestration (Hess *et al.*, 1981) and these changes might be expected to lead to the development of arrhythmias (Clusin *et al.*, 1982). But there is a little evidence for the role of oxygen free radicals in provoking reperfusion induced arrhythmias. Therefore it seemed worthwhile to

see if they are generated on reperfusion of ischaemic myocardium and if they contribute to reperfusion arrhythmias.

1.12: Washout of catecholamines and membrane phospholipid degradation products

Catecholamines are washed out during reperfusion of the ischaemic myocardium *in vivo* (Riemersma, 1982) and *in vitro* (Rochette *et al.*, 1980; Abrahamsson *et al.*, 1983). This enhanced outflow of catecholamines may be induced by reperfusion itself or may simply represent washout of catecholamines released during ischaemia. The latter possibility is partly supported by the observations that the highest release of catecholamines was during the first minute following reperfusion and then declined rapidly (Abrahamsson *et al.*, 1983; Schömig *et al.*, 1984) and that the omission of calcium from the perfusate could not abolish this increased catecholamine release (Abrahamsson *et al.*, 1984). On the contrary, Nayler and Sturrock (1984) have demonstrated no apparent noradrenaline release after 15 minutes ischaemia followed by 1 minute reperfusion, but after 15 minutes of reperfusion, the noradrenaline stores were depleted and they concluded that the noradrenaline that is lost during 15 minutes of ischaemia and reperfusion is mostly lost upon reperfusion. However, to what extent reperfusion itself causes the release of catecholamines from the nerve endings is still unknown.

The increased catecholamine release during reperfusion coupled with the antiarrhythmic effect of catecholamine depletors (Culling *et al.*, 1984) and some antiadrenergic drugs, in particular the α-adrenoceptor antagonists phentolamine and prazosin (Sheridan *et al.*, 1980; Benfey *et al.*, 1984) has led to the suggestion that

catecholamines and α-adrenoceptor stimulation are involved in the development of these arrhythmias (Sheridan *et al.*, 1980). However, other investigations demonstrated that prazosin failed to attenuate reperfusion induced arrhythmias suggesting that α-adrenergic mechanisms may be unimportant in the genesis of these arrhythmias and the anti-arrhythmic action of these drugs may not entirely be a consequence of their α-antagonist properties (Thandroyen *et al.*, 1983; Bolli *et al.*, 1984; Bralet *et al.*, 1985). It is possible that catecholamines releasedon reperfusion may undergo autoxidation and generate superoxide radicals which may contribute to their arrhythmogenic effects (Burton *et al.*, 1984).

Studies on reperfusion with propranolol, the most widely investigated β-adrenergic blocking agent have also yielded contradictory results (Sheridan *et al.*, 1980 Benfey *et al.*, 1984), suggesting that its beneficial effect may be mediated by its class I antiarrhythmic properties or by reducing the myocardial oxygen demand but not entirely due to its β-antagonist properties (Benfey *et al.*, 1984).

Depletion of membrane phospholipids and its washout on reperfusion of ischaemic myocardium have been implicated in membrane dysfunction, electrophysiological derangements, development of arrhythmias and cellular injury in ischaemic myocardium (Chien *et al.*, 1984; Corr *et al.*, 1984).

Lysophosphoglycerides are moieties with both hydrophobic and hydrophilic constituents. Because of their hydrophobic properties, they are readily incorporated into membranes, impairing their function (Corr *et al.*, 1982), and under certain conditions,

can act as detergents with resultant dissolution of membrane constituents including cholesterol and phospholipids (Corr *et al.*, 1984). The net loss of membrane phospholipids is dependent upon the rate of phospholipid deacylation by endogenous phospholipases and the rate of reacylation by phospholipid acyltransferase activities (Chien *et al.*, 1984).

Lysophosphatidylcholine can induce electrophysiological derangements simulating those of ischaemia (for details and other actions refer to: Corr *et al.*, 1982; Corr *et al.*, 1984).

C. Pharmacological interventions

1.13: Xanthine oxidase inhibition

Xanthine oxidase exists in mammals in two forms: a dehydrogenase and the oxidase form (Schoutsen *et al.*, 1983). In healthy tissues, this enzyme exists almost entirely as a dehydrogenase using NAD^+ as an electron acceptor which does not produce superoxide anion (Schoutsen *et al.*, 1983; Chambers *et al.*, 1985). In the rat heart, it has been demonstrated that a brief period of ischaemia causes the enzyme to be converted to an oxidase which uses molecular oxygen as the electron acceptor producing superoxide anions (Schoutsen *et al.*, 1983; Burton *et al.*, 1984; Chambers *et al.*, 1985). The oxidase form of the enzyme has been shown to be a major source of free radicals in ischaemia (De Wall *et al.*, 1971) and reperfusion (McCord, 1985) induced tissue injury. Although, McCord (1985) has hypothesized that increased cytosolic calcium concentration which may exist

during ischaemia can lead to activation of a number of calcium activated proteases responsible for the conversion of $x_D \longrightarrow x_o$, this process is poorly understood at present (Chambers *et al.*, 1985).

Allopurinol, the inhibitor of xanthine oxidase is rapidly metabolised in all living animals to oxypurinol (Nelson and Elion, 1975). Both allopurinol and its metabolite oxypurinol are competitive inhibitors of xanthine oxidase in both its dehydrogenase and oxidase forms (De Wall *et al.*, 1971; Chambers *et al.*, 1985). Allopurinol is cleared rapidly from the body but oxypurinol persists in the plasma because of active reabsorption by the kidney tubule and is then excreted slowly (De Wall *et al.*, 1971; Nelson and Elion, 1975).

The possibility that allopurinol may reduce the severity of myocardial ischaemic injury following experimental coronary occlusion was suggested as early as 1971 by De Wall and colleagues. They reported that allopurinol was able to decrease the number of arrhythmias following coronary artery ligation and also decrease the concentration of purine metabolites in the coronary sinus blood. On the contrary, Reimer and Jennings, (1985) have shown that allopurinol has no beneficial effect on reperfusion induced myocardial infarction in the anaesthetized dog. Therefore, it seemed worthwhile to study the effect of allopurinol on reperfusion induced arrhythmias in the isolated rat heart.

1.14: Free radical scavengers

Superoxide anion is eliminated by a family of enzymes which catalyze its dismutation and are called superoxide dismutases (Brawn and Fridovich, 1980). This group of enzymes are metalloproteic enzymes present in all oxygen-consuming organisms (Hertz and Cloarec, 1983). There are three kinds of superoxide dismutases, i.e. those based upon manganese, upon iron or upon both copper and zinc (Brawn and Fridovich, 1980). Their only known biological function is the dismutation of the superoxide radical, and the intracellular level of superoxide dismutase increases by exposure of organisms to oxygen (Hertz and Cloarec, 1983). The concentration of superoxide dismutase in biological cells is of the order of 10^{-6} M (Nishikimi, 1975). All kinds of superoxide dismutases catalyze the same reaction and all operate by a similar mechanism in which the metal at the active site is alternately reduced and then reoxidized during encounters with superoxide radicals (Brawn and Fridovich, 1980).

Catalases and peroxidases are specific scavengers of hydrogen peroxide (Frank, 1983). The action of catalases is an oxidation-reduction in which one molecule of hydrogen peroxide acts as a reductant towards another hydrogen peroxide molecule. The net effect is the conversion of hydrogen peroxide into water and molecular oxygen. This is an efficient way to eliminate H_2O_2, since no other reductant is needed. In contrast, peroxidases use a non-peroxide reductant to reduce peroxide. Thus, for example, glutathione peroxidase catalyzes the reaction:

$$2G\text{-}SH + H_2O_2 \longrightarrow GS\text{-}SG + 2H_2O$$

This is less efficient in that glutathione (G-SH) is used up and must be regenerated (Brawn and Fridovich, 1980).

The last class of specific free radical scavengers does not exist in biological cells as a defense mechanism and includes mannitol and histidine (Lynch and Fridovich, 1978; Frank, 1983; Magovern *et al.*, 1984). Mannitol, in addition to being used as a hyperosmolar agent has been shown to be an effective scavenger of the cytotoxic hydroxyl radical (Hess *et al* ., 1981; Jolly *et al.*, 1984; Magovern *et al.*, 1984). This effect may be due to the interaction of the aldehyde moiety of mannitol with the hydroxyl radical resulting in reduction of the free radical and formation of mannitol free radicals which are less toxic to biological cells (Magovern *et al.*, 1984). Histidine has also been reported to be a specific quencher of singlet oxygen (Lynch and Fridovich, 1978; Brawn and Fridovich, 1980) which may be generated through the interaction between O^{-}_{2} and H_2O_2 (Lynch and Fridovich, 1978).

Furthermore, several antioxidants like ascorbic acid, α-tocopherol, cysteine and reduced glutathione may have free radical scavenging effects (Burton *et al.*, 1984). Ascorbic acid is present in rather high amounts in both animal and plant tissues, its concentration in biological cells is of the order of 10^{-3} M (Nishikimi, 1975). Ascorbic acid has been indicated to have a free radical scavenging ability according to the following sequence (Nishikimi, 1975):

$$O_2^{\cdot} + AH_2 + H^{+} \longrightarrow H_2O_2 + \dot{A}H$$

$$O_2^{\cdot} + \dot{A}H + H^{+} \longrightarrow H_2O_2 + A$$

Members of the intracellular defense mechanism including dismutase, catalase, glutathione peroxidase and ascorbic acid have been shown to decrease during myocardial ischaemia (Rao *et al.*

1983; Jolly *et al.*, 1984), this has suggested the use of some exogenous free radical scavengers and antioxidants to replenish the myocardium with protective agents against the expected tissue damage by free radicals which may result due to the deficit in the defense mechanisms or the increased production of free radicals expected on reperfusion of ischaemic myocardium.

1.15: Agents affecting arachidonic acid metabolism

Arachidonic acid is a polyunsaturated fatty acid with a 20-carbon backbone and four double bonds. When released from phospholipids by specific phospholipases, free arachidonic acid immediately undergoes either enzymatic conversion by cyclo-oxygenase to endoperoxide intermediates which are converted to prostaglandins or thromboxanes, or enzymatic conversion by lipoxygenases to hydroperoxy acid intermediates which form leukotrienes (Weksler, 1984).

Prostaglandin synthesis involves two stages, cyclo-oxygenation followed by polymerization or reductive cleavage of the endoperoxides (Higgs and Vane, 1983). In the endothelial or smooth muscle layers of the blood vessel wall, arachidonic acid is preferentially converted via cyclo-oxygenase to prostacyclin (PGI_2), a potent vasodilator that inhibits platelet aggregation (Bunting *et al.*, 1983; Weksler, 1984). In contrast, in blood platelets, spleen, kidney and lung tissues, arachidonic acid is preferentially converted by cyclo-oxygenase and thromboxane synthetase to thromboxane A_2, a powerful vasoconstrictor and inducer of platelet aggregation (Whittle and Moncada, 1983; Weksler, 1984).

Lipoxygenases are a group of iron containing enzymes found in blood platelets, polymorphonuclear leucocytes and in many organs including lung, pituitary and vascular tissues (Taylor and Morris, 1983). Leukotrienes have been reported to cause a marked reduction in coronary flow with decrease in contractility and reduction in spontaneous heart rate in rat hearts (Piper, 1983). Fig. (3) shows schematic representation of the two pathways of arachidonic acid metabolism.

There are a number of compounds available which interfere with prostaglandin and leukotriene synthesis. The glucocorticoids have been reported by Blackwell and Flower (1983) to have no direct effect upon phospholipase A_2 but interact with specific glucocorticoid receptors leading to the secretion and synthesis of proteins (macrocortins) that possess antiphospholipase properties. Non steroidal anti-inflammatory drugs like aspirin and indomethacin inhibit the cyclo-oxygenase step of arachidonic acid metabolism (Higgs and Vane, 1983). Imidazole compounds can selectively block the generation of thromboxane A_2 by thromboxane synthetase from the cyclic endoperoxides without suppressing prostacyclin production by the vessel wall (Bunting *et al.*, 1983).

The vasoconstrictor actions of leukotrienes on the coronary circulation suggest that they may be involved in conditions such as myocardial ischaemia and are generated locally in the coronary vascular bed (Piper, 1983). Furthermore, the release of prostaglandins has been reported to be increased during myocardial ischaemia (Coker, 1982). However, there is some controversy regarding the effects

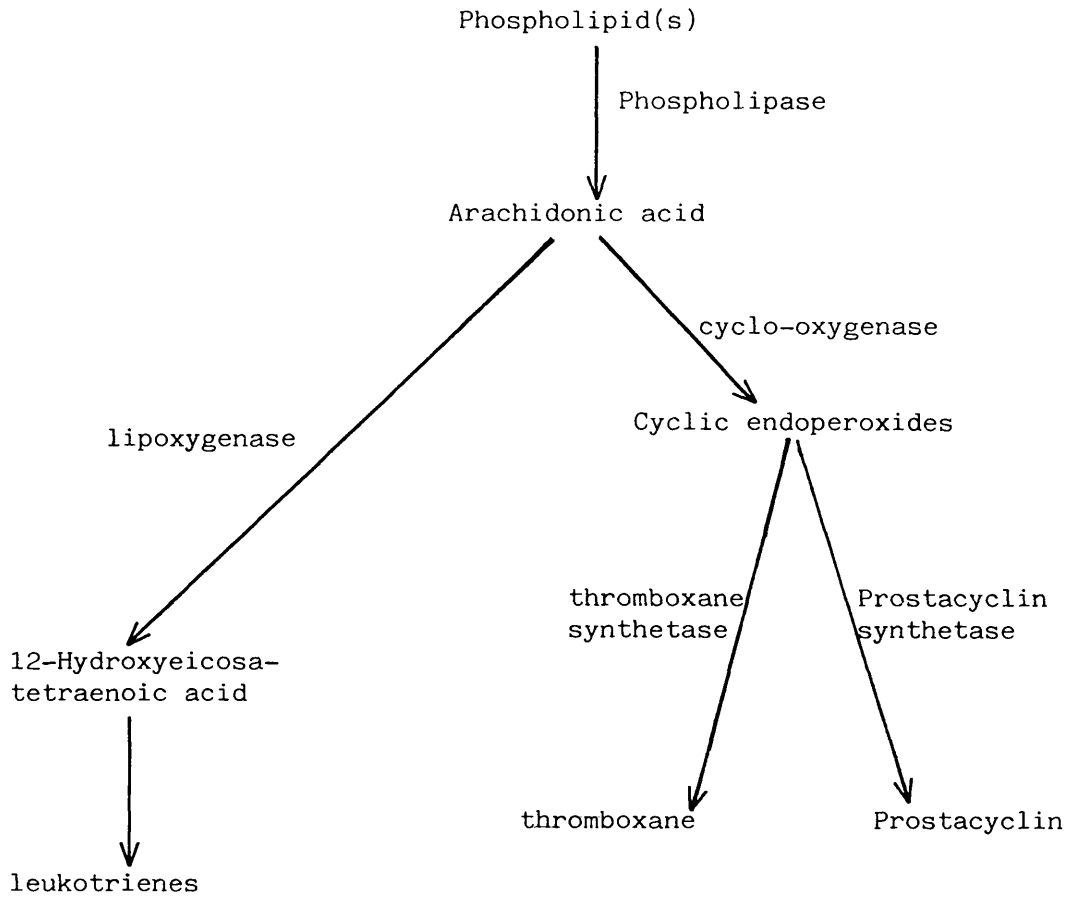


Fig. (3). Enzymatic conversion of arachidonic acid to prostaglandins and leukotrienes.

of prostaglandins and their synthesis inhibitors on arrhythmias resulting from coronary artery occlusion (For details see Coker, 1982). Therefore, it seemed worthwhile to investigate the effect of some inhibitors of arachidonic acid metabolism on reperfusion induced arrhythmias.

Aim of the Present Investigation

- The aim of this study was to develop a model for reliable and reproducible production of reperfusion induced arrhythmias in the isolated rat heart which elicits a high incidence of these arrhythmias.

- To study the effect of the rate of coronary flow and rate of reperfusion on the severity of the resulting arrhythmias.

- To investigate the influence of the ionic environments of the heart on the incidence of reperfusion arrhythmias and to try to find out the possible mechanisms of action of calcium, magnesium and potassium.

- To study the possible role of oxygen free radicals in reperfusion arrhythmogenesis and cell membrane damage as indicated by the increase in $^{86}Rb^{+}$ efflux rate constant. This can be carried out by:-

  a) Investigating the effects of free radical scavengers on reperfusion induced arrhythmias.
  b) Detection of superoxide radicals in the perfusate of the isolated rat heart.
  c) Investigating the effects of oxygen free radicals and oxygen free radical scavengers on the efflux rate constant of $^{86}Rb^{+}$.

- To study the effect of some agents which affect arachidonic acid metabolism and some vasodilating agents on reperfusion induced arrhythmias in the isolated rat heart.

CHAPTER 2

MATERIALS AND METHODS

2.1: Experimental Protocol

1. Induction of regional ischaemia and reperfusion *in vitro*

Hearts from male Wistar rats (200 to 300 g University of Bath strain) were perfused retrogradely via the aorta at a constant flow of 10 ml/min and 37°C with a physiological solution of the following composition (mM) NaCl 118; KCl 4.7; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25; $CaCl_2$ 1.23 and glucose 11 gassed with 95% O_2, 5% CO_2. A loose ligature was immediately placed round the main left coronary artery, both ends of the ligature were then passed through a short piece of polythene tubing 1 mm i.d. to form a snare. Following a 15 minutes equilibration period the composition of the perfusate was changed according to the aim of the experiment. In most of the experiments the potassium concentration of the perfusate was reduced to 3.2 mM, as low concentrations of potassium enhance the development of reperfusion-induced arrhythimas in the isolated rat heart (Lubbe *et al.*, 1978; Woodward and Zakaria, 1983). This was achieved by reducing the concentration of KCl from 4.7 to 2.0 mM while maintaining the concentration of KH_2PO_4 at 1.2 mM. Five minutes later the drug under investigation was added to the perfusate and five minutes later the snare around the coronary artery was tightened and held in place with a small clip. An increase in perfusion pressure indicated successful ligation. Ten minutes later the clip holding the ligature was removed, a decrease in perfusion pressure indicated successful reperfusion. Fig. (4) shows the perfusion protocol used. Epicardial

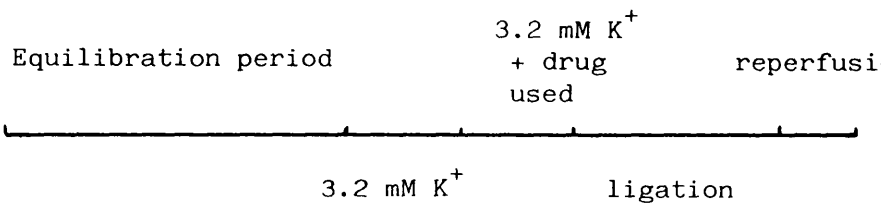


Fig. (4). The protocol of induction of regional ischaemia and reperfusion in the isolated perfused rat heart.

ECGs were recorded on a Devices MX2 recorder, the recording electrodes being placed on the right atrium and left ventricle. As reperfusion arrhythmias are rapid in onset, ECGs were usually recorded for 3 min following reperfusion, however, in some experiments reperfusion was continued for 10 min when no serious arrhythmias occurred within 3 minutes (see Results). Developed tension at a diastolic tension of 2 g was recorded via a Devices UF1 isometric transducer attached to the tip of the left ventricle. This tension recording was used to trigger a Devices rate meter (4521) to monitor heart rate. Perfusion pressure was recorded with a Bell Howell pressure transducer (4-442). Recordings were made on Devices M19 recorder. Developed tension was also recorded at a fast chart speed on the second channel of the MX2 recorder. In some experiments hearts were paced electrically using a Grass S48 stimulator. Pacing electrodes were placed on the A-V groove and the metal aortic cannula, stimulation parameters were square wave pulses 2 m sec duration, 5 Hz at a supramaximal voltage. In a preliminary series of experiments, it was determined that a 10 min occlusion period was optimal for the development of reperfusion arrhythmias. Fig. (5) shows diagrammatic representation of the perfusion apparatus. And Fig. (6) shows traces of perfusion pressure, developed tension and heart rate recordings during the time course of 10 min ischaemia and reperfusion in the isolated rat heart.

2. Induction of global ischaemia and reperfusion

In these experiments no ligature was placed around the left descending coronary artery. Global ischaemia was induced by reducing the flow rate from 10 to 0 $ml.min^{-1}$ by switching off the perfusion

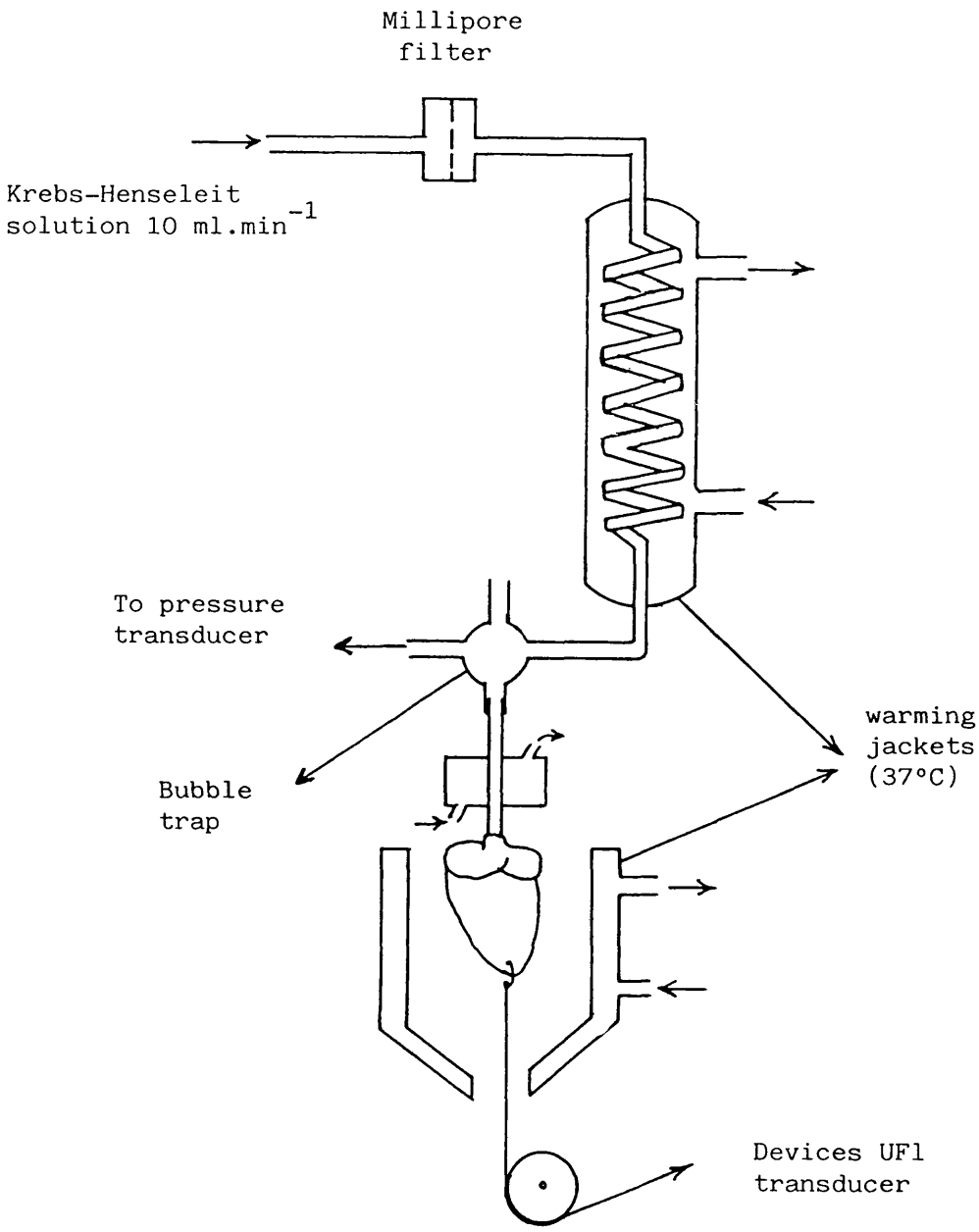


Fig. (5) Diagrammatic representation of perfusion apparatus.

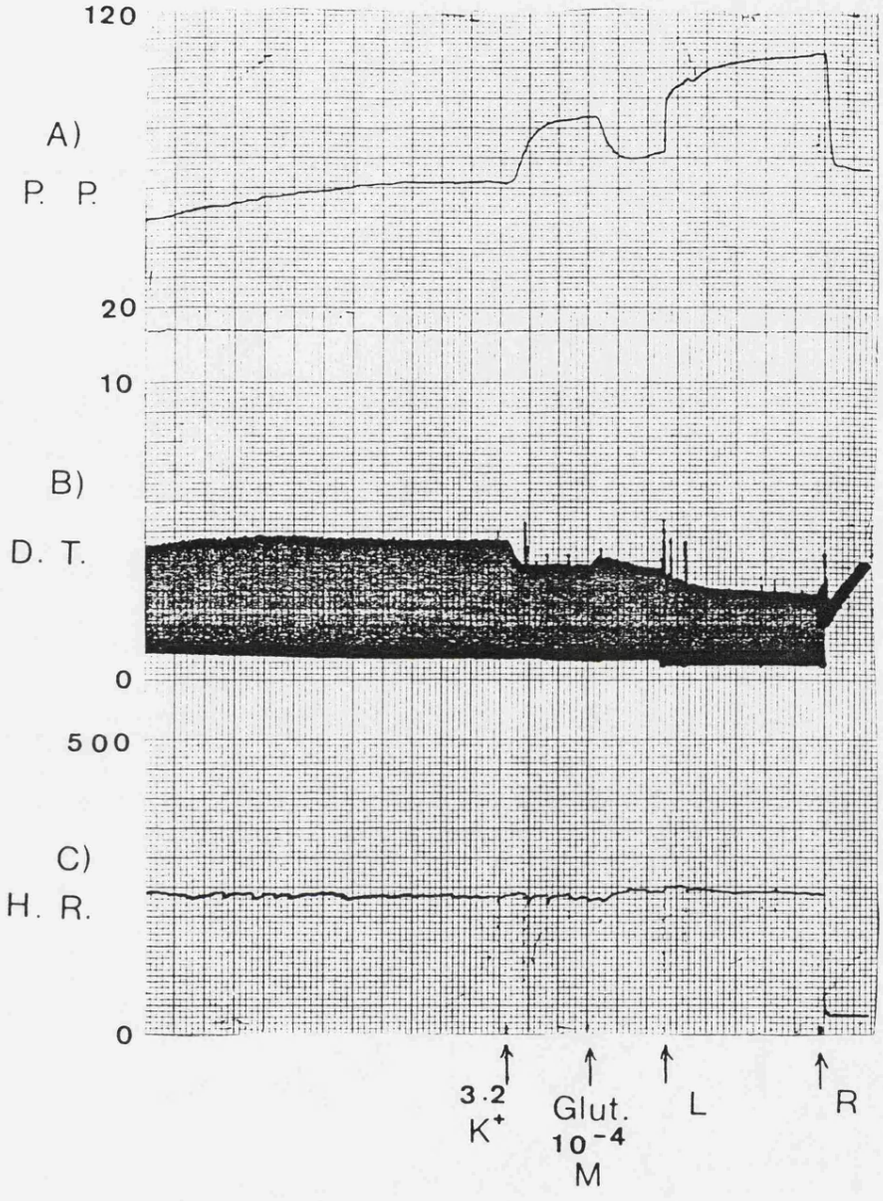


Fig. (6). Traces of (A) perfusion pressure (mm Hg) (B) developed tension (g) and (C) heart rate (beats.min^{-1}) recordings during the time course of 10 minutes of regional ischaemia and reperfusion in the isolated rat heart.

pump for ten minutes then reperfusion started by reswitching on the perfusion pump.

3. Perfusion under a constant head pressure

Some hearts were perfused under a constant head pressure (100 cm H_2O) by keeping the level of the surface of the perfusate always 100 cm above the level of the heart.

4. Reoxygenation of anoxic myocardium

Ten minutes anoxia were induced using perfusate of the same composition as previously described but gassed with 95% N_2, 5% CO_2, then ten minutes later reoxygenation was achieved by the use of perfusate gassed with 95% O_2, 5% CO_2. In these experiments anoxia and reoxygenation were induced instead of ischaemia and reperfusion.

5. Induction of ischaemia and reperfusion *in vivo*

Rats were anaesthetized with pentobarbitone sodium 60 $mg.kg^{-1}$ intraperitoneally, with small additional amounts administered intravenously as required. Systemic arterial blood pressure was recorded from the left common carotid artery using a Bell Howell pressure transducer (4-442). A catheter was placed in a femoral vein for administration of drugs, and the trachea was cannulated to allow artificial ventilation. Arterial blood pressure and a standard lead II electrocardiogram were recorded on a Devices MX2 recorder. Rectal temperature was maintained at approximately 38°C. The chest was opened by left thoracotomy at the fifth intercostal space and the fifth and fourth ribs were sectioned approximately 2 mm from the left margin of the sternum. Immediately after opening the chest,

the animals were ventilated with room air using a stroke volume of about 2 ml/100 g and a rate of 54 strokes/min which maintain arterial PO_2, PCO_2 and PH within the normal range. After opening the pericardium, the heart was exteriorized by gentle pressure on the chest walls and a ligature was placed under the left coronary artery (Clark *et al.*, 1980), both ends of the ligature were then passed through a short piece of polythene as described before. The heart was replaced in the chest cavity and any animal in which this procedure itself produced dysrhythmias or a sustained fall in mean arterial blood pressure to less than 70 mm Hg was excluded from the study at this point. After 15 minutes equilibration period the ligature was tied and held in place with a small clip for 10 minutes ligation period, then the clip was removed to achieve reperfusion.

6. Induction of arrhythmias by perfusing with arrhythmogenic solution

Hearts were made to fibrillate in the absence of coronary ligation and reperfusion by perfusing with an arrhythmogenic solution which differed from the normal perfusate in that KCl and $MgSO_4$ were reduced to 1.2 mM and 0 mM respectively while the $CaCl_2$ level was increased from 1.23 mM to 4.92 mM. This perfusate caused VF after 255 ± 10 sec (n=5).

2.2 Criteria for arrhythmias

Premature ventricular contractions (PVCs) were clearly seen from the ECG and developed tension traces recorded on the MX2 recorder. Isolated PVCs were followed by a compensatory pause while runs of PVCs which constituted ventricular tachycardia (VT) always had

smaller contraction amplitudes than normal.

Ventricular tachycardia (VT) was diagnosed as five or more consecutive PVCs. Ventricular fibrillation (VF) was diagnosed when the ECG recording showed chaotic activity with an amplitude less than that of the normal ECG. The total PVC number and the incidence, onset and duration of VF and VT were recorded. Fig. (7) shows ECG and developed tension recorded during the incidence of VT and VF on reperfusion following 10 minutes ischaemia.

2.3:Ferricytochrome C reduction

In some experiments hearts were perfused with ferricytochrome C (5×10^{-5} M). Its reduction after passing through the heart was measured at a wave-length of 550 nm in a Hilgar Gifford spectrophotometer (Goldstein *et al.*, 1975).

2.4: Protein determination

Determination of protein in perfusate was done by the Lowry method (for details refer to Lowry *et al.*, 1951). Fig. (8) shows typical calibration curve for protein.

2.5: Lactate determination

Principle of assay

The basic equation for the spectrophotometric assay of L-lactate was:

$$\text{Lactate} + \text{NAD}^+ + \text{Hydrazine} \xrightarrow[\text{PH 9.5}]{\text{LDH}} \text{Pyruvate hydrazone} + \text{NADH} + \text{H}^+$$

LDH : Lactate dehydrogenase

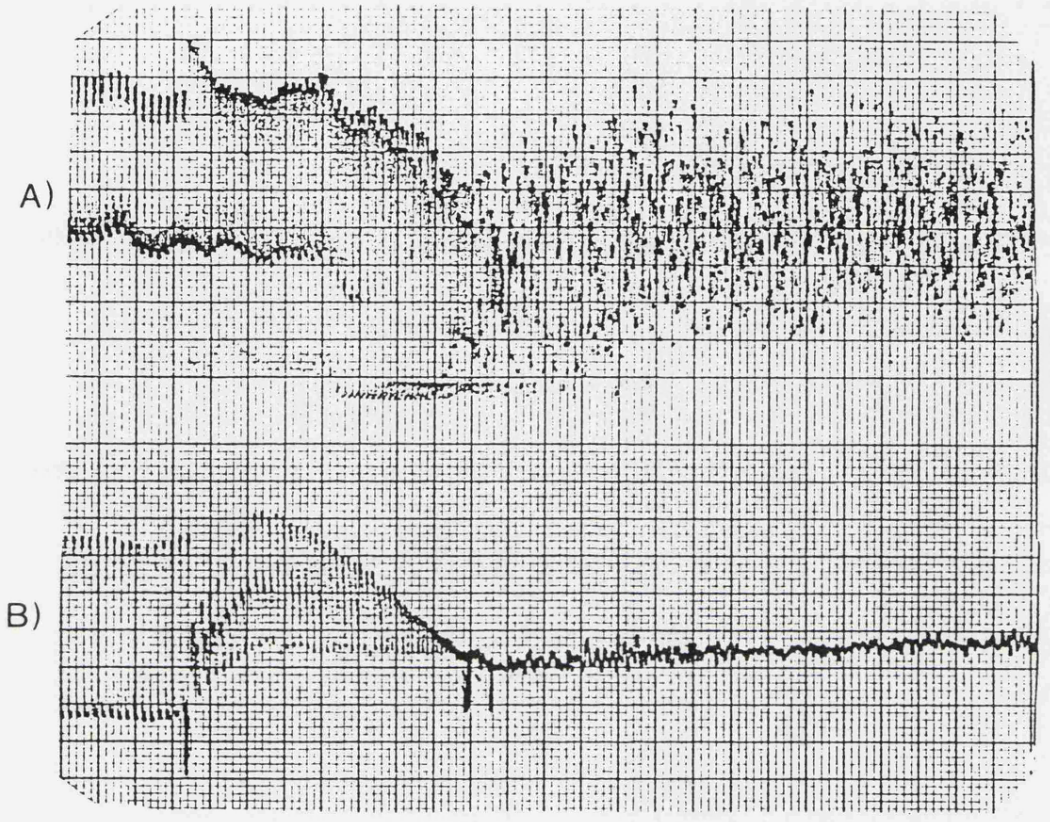


Fig. (7). (A) ECG and (B) developed tension traces recorded on MX2 recorder showing the incidence of VT and VF on reperfusion after 10 minutes ischaemia in the isolated perfused rat heart.

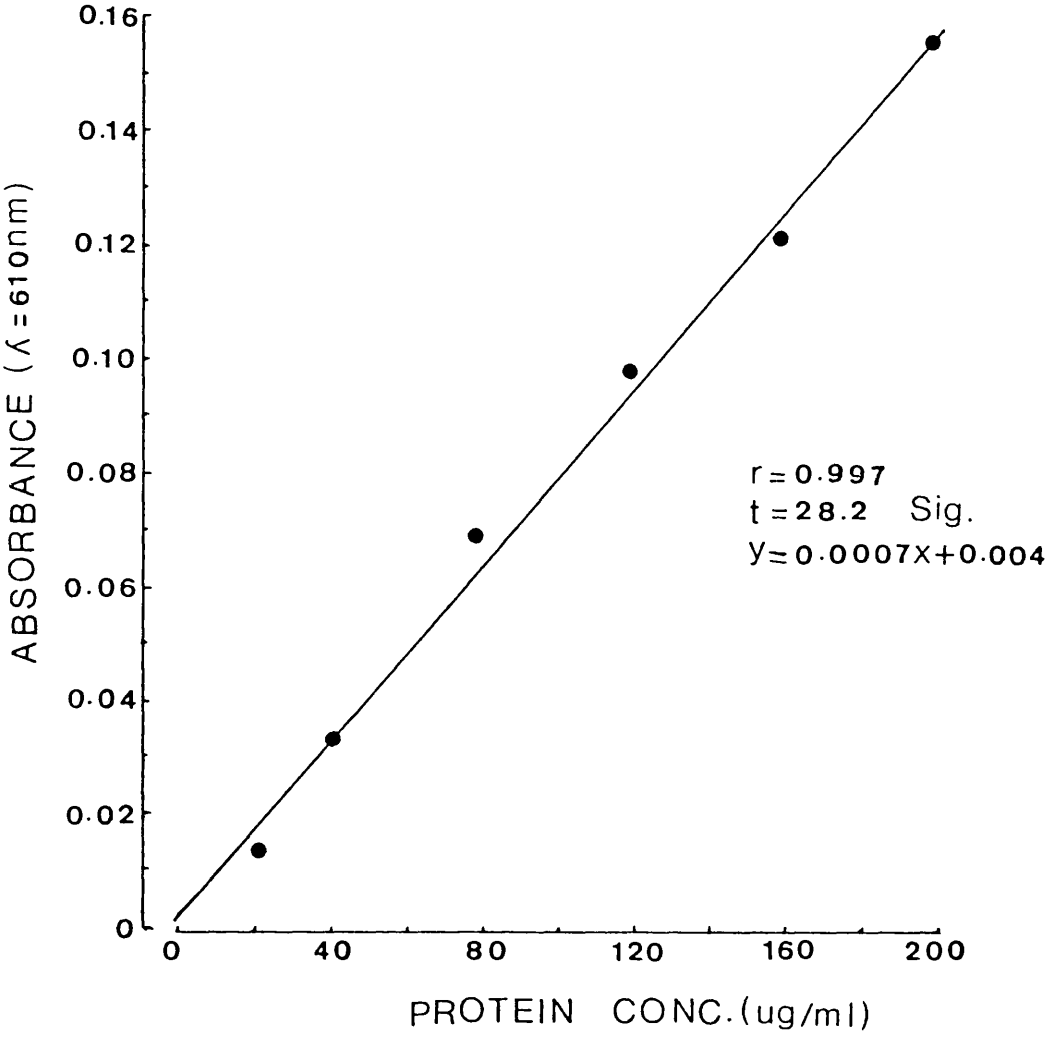


Fig. (8). Typical calibration curve for protein.

Therefore, the course of the reaction is followed spectrophotometrically by the increase in optical density due to the formation of NADH.

Assay procedure

Lactate was determined by a method similar to that described by Hohorst (1965). The following reagents were used:

(a) Hydrazine (1.25 M) - glycine (2M) buffer(containing 20 mM EDTA with pH adjusted to 9.5 with 2 M NaOH).

(b) Nicotinamide adenine dinucleotide (oxidised) 0.05 M.

(c) LDH (from rabbit skeletal muscle - Boehringer Cat. No. 127230) solution diluted with distilled water to give 0.5 $ng.ml^{-1}$.

0.45 ml hydrazine buffer, 0.50 ml distilled water and 0.05 ml NAD^+ were added to the cuvette, then 0.1 ml perfusate or distilled water (blank) was added, mixed thoroughly. The cuvette contents were allowed to warm to room temperature and the optical density was read at wavelength 340 nm twice with an interval of 3 min. LDH suspension was added and mixed into the experimental cuvette. On completion of the reaction, the optical density was read twice E_1 and E_2 ($\Delta E = E_2 - E_1$, was very small or zero)

Calculations: Extinction coeff. $NADH_{340} = 6.22 \times 10^{-3}$

$$O.D. \times 6.22 \times 10^{-3} = 1 \text{ M}$$

The total volume = 1.105 ml

$$\text{Lactate content} = \frac{O.D. \times 1.105}{6.22 \times 1000} \text{ m Moles}$$

$$= \frac{O.D. \times 1.105}{6.22} \text{ } \mu \text{ Moles (in samples of 0.1 ml)}$$

$$\text{Lactate content } (\mu\text{Moles/ml}) = \frac{O.D. \times 1.105}{6.22} \times 10$$

2.6: Malondialdehyde determination in heart tissue

Heart homogenate (10% w/v) was prepared in 0.2 M Tris-0.16 M KCl buffer of pH 7.4 and incubated for 1 hour at 37°C in a water bath. 1 ml aliquot was withdrawn from the incubation mixture and pipetted into a pyrex tube. This was followed by addition of 0.5 ml of 40% trichloroacetic acid and 0.25 ml of 5 N HCl. After mixing, 0.25 ml of 2% sodium α-thiobarbiturate was added. The tubes were boiled for 15 min and cooled in a bucket of ice. One ml of 70% trichloroacetic acid was then added and tubes were allowed to stand for 20 min, centrifuged at 2500 r.p.m. for 10-15 minutes and the colour read at a wavelength of 532 nm. The standard tubes contained 1 nMoles of MDA (Singal *et al.*, 1983). Fig. (9) shows typical calibration curve for MDA.

2.7: Determination of vitamin E in serum

Serum samples (0.2 ml) or distilled water or standard vitamin E were put in very clean glasses and were diluted with 1 ml distilled water. Then 0.4 ml of methanol and 0.6 ml of hexane were added to each sample. They were then mixed very quickly for 30 seconds. Samples were centrifuged at 1000 r.p.m. for 10 min. Vitamin E was present in the hexane phase (upper layer). These extracts were read on the spectrophotofluorimeter at excitation wavelength 295 nm and emission wavelength 330 nm.The values corresponding to each sample were obtained by extrapolation on a calibration curve plotted with standard Vit E (Fig. (10)) (Falanga *et al.*, 1983).

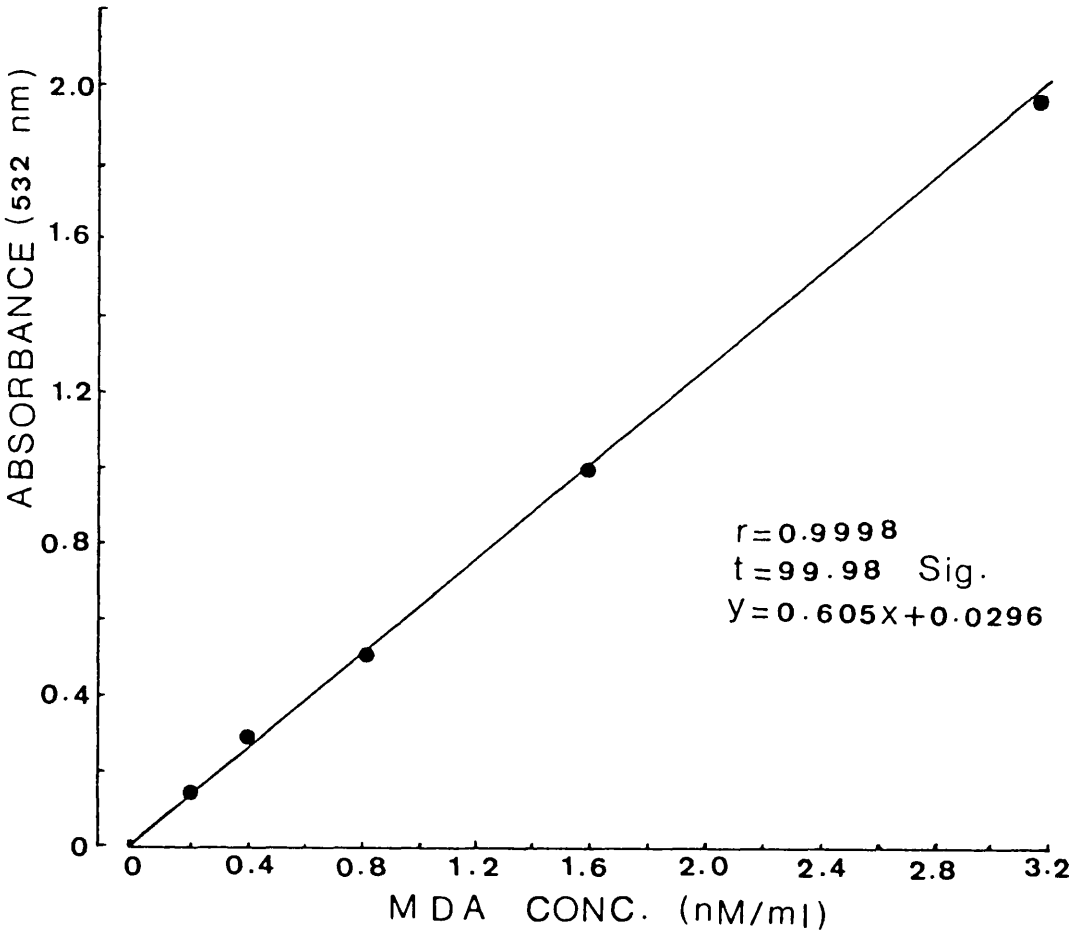


Fig. (9). Calibration curve for malondialdehyde.

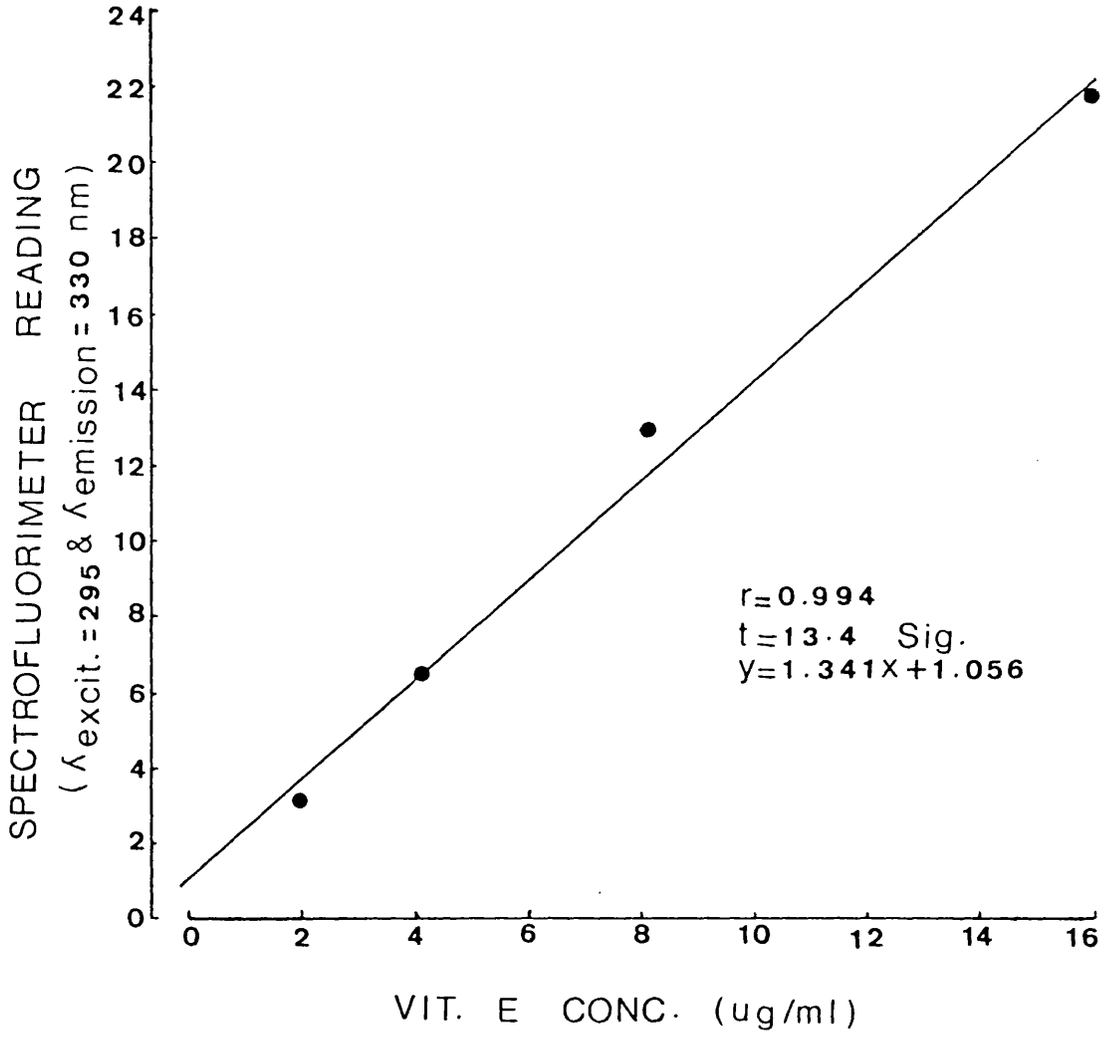


Fig (10). Typical calibration curve for Vit. E.

2.8: Determination of $^{86}Rb^{+}$ efflux rate constant by liquid scintillation counting

A) Experimental Protocol

After a 5 min equilibration period as described at (2.1), hearts were loaded with $^{86}Rb^{+}$ (0.2 μCi/ml) for 10 minutes, then washed for 30 minutes period by perfusion with Krebs solution and a sample was collected every minute during the last 15 min representing the control samples while samples of the first 15 min were discarded. Hearts were then perfused with Krebs solution with changed K^{+} concentration and five minutes later, the drug under investigation was included in the perfusate. Five minutes later, the left descending coronary artery was ligated for 10 minutes and then the ligature was released for 3 minutes. A sample was collected every minute over the whole experiment except on reperfusion when samples were collected every 30 seconds.

B. Treatment of samples

4 ml of scintillation cocktail were added to 1 ml of perfusate solution, shaken and counted in the liquid scintillation counter (LKB 1215).

Heart tissue samples were dissolved in 5 ml of 1 N KOH for 48 hours, neutralized with 5 ml of 1 N HCl. 1 ml of the neutral solution was added to 4 ml scintillation cocktail, shaken and counted in the liquid scintillation counter (Durbin and Jenkinson, 1961).

C) Treatment of results

Efflux rate constants were calculated by dividing counts in the perfusate by the number of counts contained in the heart during the collection period:

$$\text{Efflux rate constant.min}^{-1} = \frac{\text{counts in perfusate}}{\text{tissue count x collection time (min)}}$$

N.B.: Tissue count = the counts in perfusate after this minute plus counts found in the heart at the end of the experiment.

D) Construction of quench curve

A quench curve was constructed by the use of the external standard channel ratio as follows:

Four ml of scintillation cocktail (optaphase safe) and 0.02 µCi of $^{86}Rb^{+}$ were added to 10 mini-vials. Quantities of 20, 40, 60, 80, 100, 120, 140, 160 and 180 µl of CCl_4 were added to tubes 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. Then the volume was completed to 5 ml with distilled water to simulate the emulsion system found in the experimental conditions, then the tubes were capped and shaken.The activity in 0.02 µCi of $^{86}Rb^{+}$ was counted in terms of dpm and entered in the counter. Samples were then loaded into the LKB 1215 liquid scintillation counter which had been programmed to count the samples and to construct automatically a quench curve (Fig. 11). This curve was stored in the counter's memory so that dpms for all subsequent samples were calculated automatically.

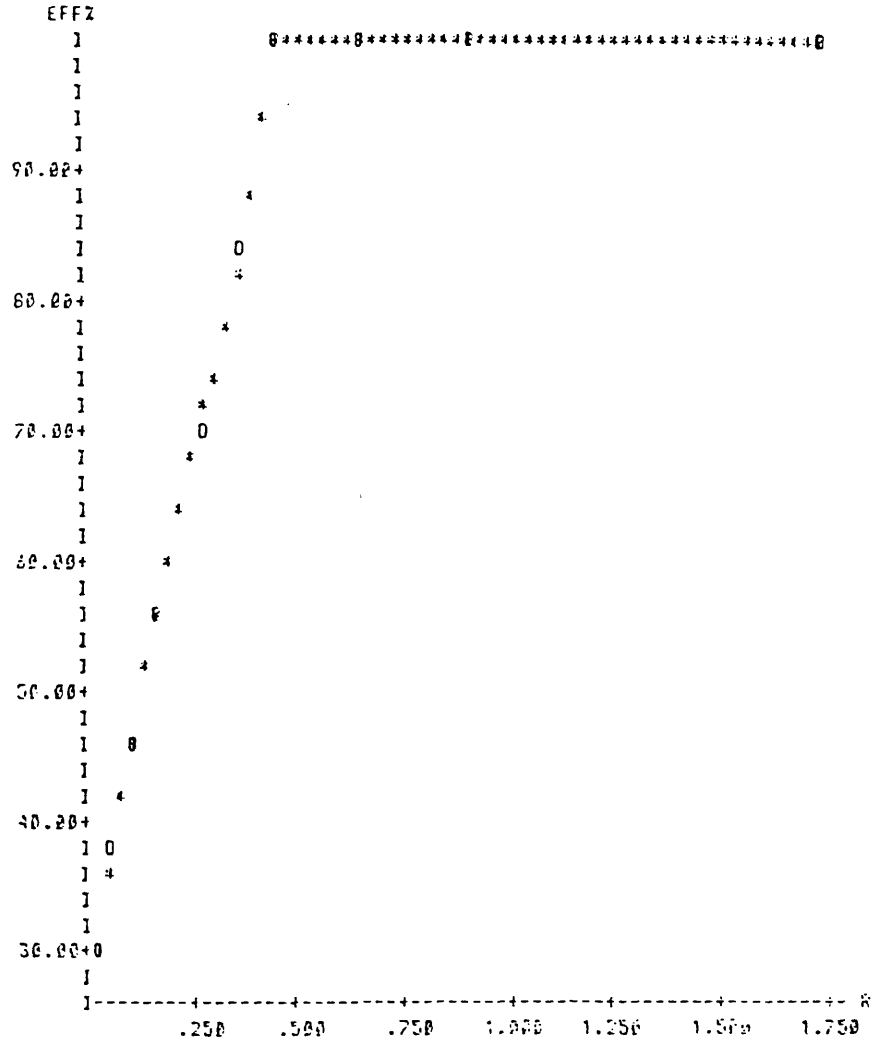


Fig. (11). Quench curve for $^{86}Rb^{+}$ determined by LKB 1215 liquid scintillation counter.

2.9: Determination of ^{3}H-NA release by liquid scintillation counting

The method and calculation used are the same as in Chapter 2.8, except in that the heart was loaded with 0.17 μCi/ml (instaed of 0.2 μCi/ml) for the same period of time (10 minutes). Fig. (12) shows the quench curve for tritium.

2.10: Statistical methods

Results are presented as mean ± S.E.M. The χ^2 test was used to compare the incidence of VT and VF in control and drug treated groups. The Wilcoxon Rank Sum test was used to compare PVC numbers and the onset and duration of VT and VF. Students t-test was used to compare changes in perfusion pressure, developed tension, heart rate and the other biological measurements. $P < 0.05$ was considered to be significantly different from control values.

2.11: Materials used

Adenosine	Boehringer and Soehne GmbH Mannheim
Allopurinol	Sigma
Ascorbic acid	BDH
Aspirin	Sigma
BW 755C	Wellcome
N-butylimidazole	Koch Light Labs
Catalase	Sigma
Dazoxiben	Pfizer
Desferrioxamine	Ciba Labs
Dexamethasone	Sigma
Dimethylacetamide	Koch Light Labs

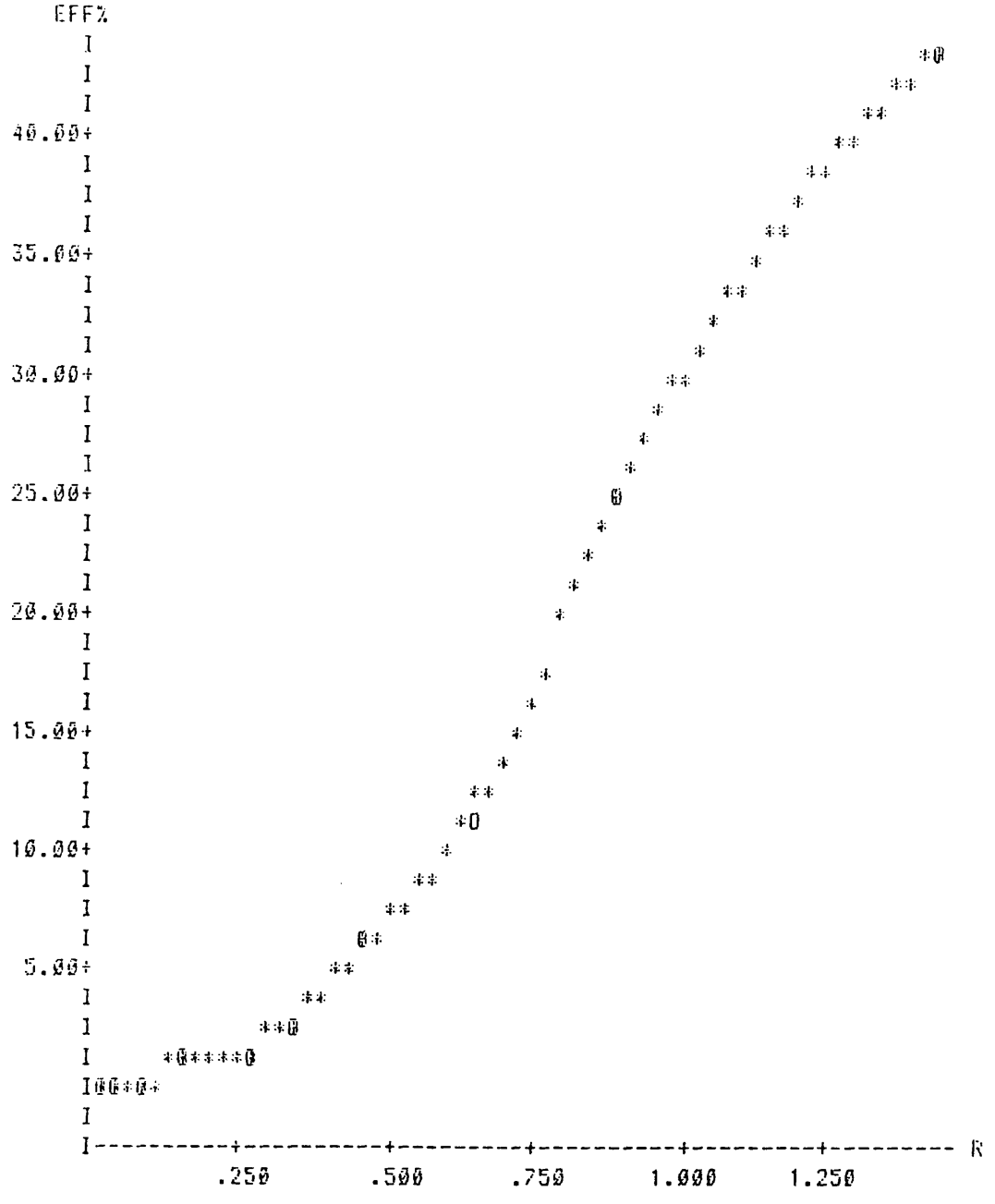


Fig. (12). Quench curve for tritium determination by the LKB 1215 liquid scintillation counter.

Ferricytochrome C (from horse heart type III)	Sigma
Ferrous chloride	BDH
Glutathione	Sigma
Histidine	Sigma
Hydrogen peroxide	Fisons
6-Hydroxydopamine	Sigma
Indomethacin	MSD
Lactate dehydrogenase	Boehringer and Soehne GmbH Mannheim
Malondialdehyde	BDH
Mannitol	BDH
Nicotinamide adenine dinucleotide	Boehringer and Soehne GmbH Mannheim
^{3}H-Noradrenaline	Amersham
Pentobarbitone sodium	M&B Vet. products
^{86}Rubidium	Amersham
Sodium nitroprusside	Sigma
Superoxide dismutase (from bovine heart)	Sigma
Thymol	Sigma
Verapamil HCl	Abbott
Vitamin E	BDH
Xanthine	Sigma
Xanthine oxidase	Sigma
Z.K. 36374	Schering

CHAPTER 3

"Effect of the preceding period of ischaemia and the model of induction of reperfusion on the severity of the developing arrhythmias"

Section A: Results

3.1: Determination of the optimal occlusion period for the development of reperfusion arrhythmias

It was important to start with determination of the optimal occlusion period which induces the highest incidence of reperfusion arrhythmias in general and VF in particular in order to guide the extension of work in this thesis. Fig. (13) shows a bell-shaped relationship between number of PVCs developing on reperfusion and the period of preceding ischaemia with highest development of PVCs on reperfusion after 15 to 20 minutes of ischaemia. Fig. (14a) shows a gradual increase in VT incidence with increasing the preceding ischaemic period until it reached 100% on reperfusion after 15 min or longer periods of ischaemia. As in Fig. (14b), the shortest onset of VT existed after 10 minutes and increased by increasing the ischaemic period. The longest duration of VT occurred after 10 minutes of ischaemia and decreased by increasing the ischaemic period (Fig. 14c). Fig. (15 a,b) shows a highest incidence and shortest onset of VF on reperfusion after 10 min of ischaemic period. Duration of VF was found to be higher after 10 min, then decreased by increasing the ischaemic period until 20 min and increased again until it reached the maximum after reperfusion after 30 minutes of ischaemia (Fig. (15c)). Therefore, 10 min is the optimal occlusion period for the highest incidence of reperfusion induced arrhythmias.

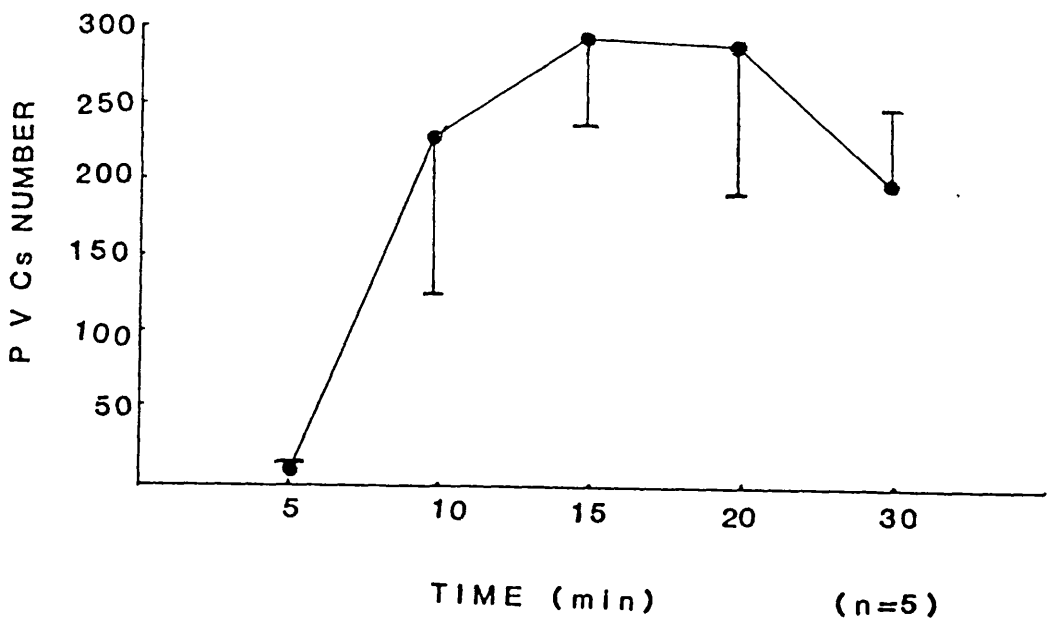


Fig. (13). Effect of the length of the preceding ischaemic period on the number of PVCs developed during reperfusion.

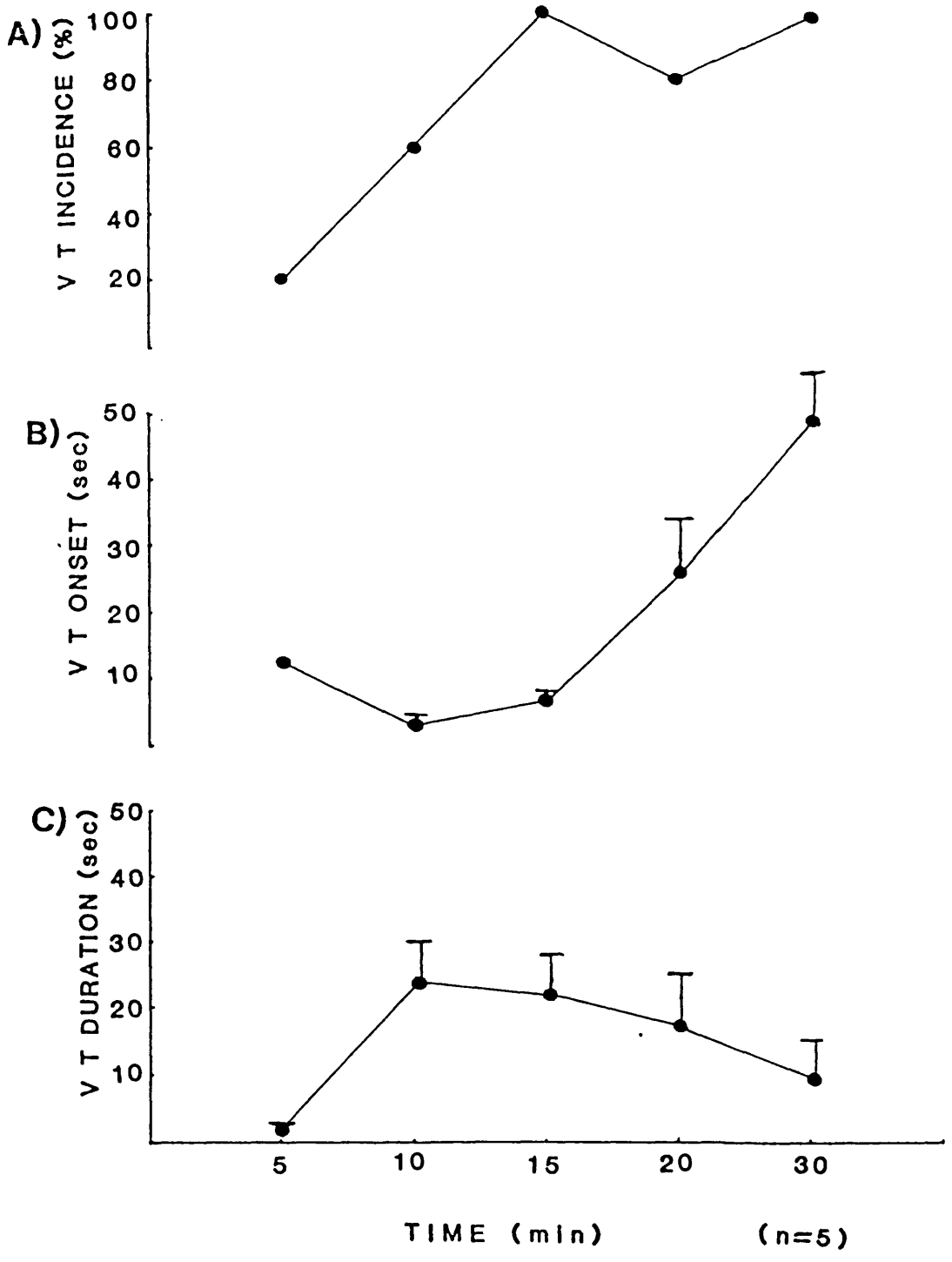


Fig. (14). Effect of the preceding period of ischaemia on:

A) VT incidence(%)

B) VT onset (sec)

C) VT duration (sec)

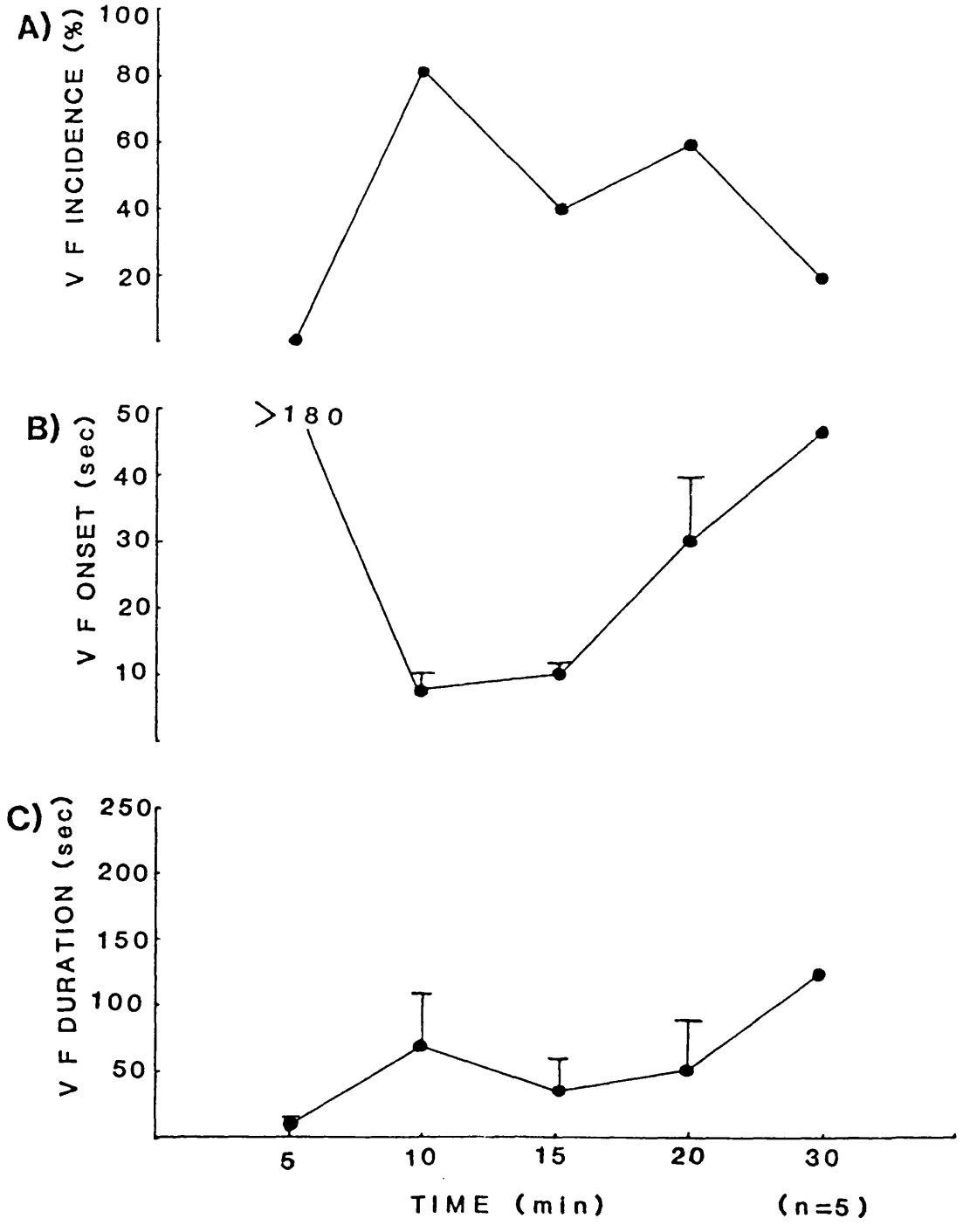


Fig. (15). Effect of the preceding period of ischaemia on:

A) VF incidence (%)

B) VF onset (sec)

C) VF duration (sec)

3.2: Comparison of reperfusion arrhythmias induced in some different rat heart models

After determination of the optimal occlusion period for reperfusion arrhythmias as being 10 minutes it was logical to find out if the incidence of reperfusion arrhythmias after regional ischaemia using a constant flow rate differed from that occurring on reperfusion of globally ischaemic hearts or that occurring under constant head of pressure, or that occurring *in vivo*.

Fig. (16) shows that the number of PVCs occurring on reperfusion of ischaemic myocardium *in vivo* was more than three times higher than that on reperfusion *in vitro* after regional ischaemia and more than that on reperfusion after global ischaemia either in absence or presence of pacing (used to prevent the dramatic decrease in heart rate). PVCs numbers in both reperfusion after regional ischaemia under constant flow rate and after regional ischaemia under constant head pressure were approximately similar. The incidence of VT was high and similar in the three models of regional ischaemia while the incidence of VT was zero on reperfusion after global ischaemia (Fig. (17a)). Furthermore, no variation in VT onset (Fig. (17b)) existed among the three models of regional ischaemia but the duration of VT was 2½ times longer in the *in vivo* model than those in the *in vitro* models (Fig. (17c)). Pacing of the globally ischaemic heart (at 250 $b.min^{-1}$) had little effect on VT developed on reperfusion. In Fig. (18a) it can be seen that VF incidence was slightly higher in the model of regional ischaemia under constant flow rate than that in the other

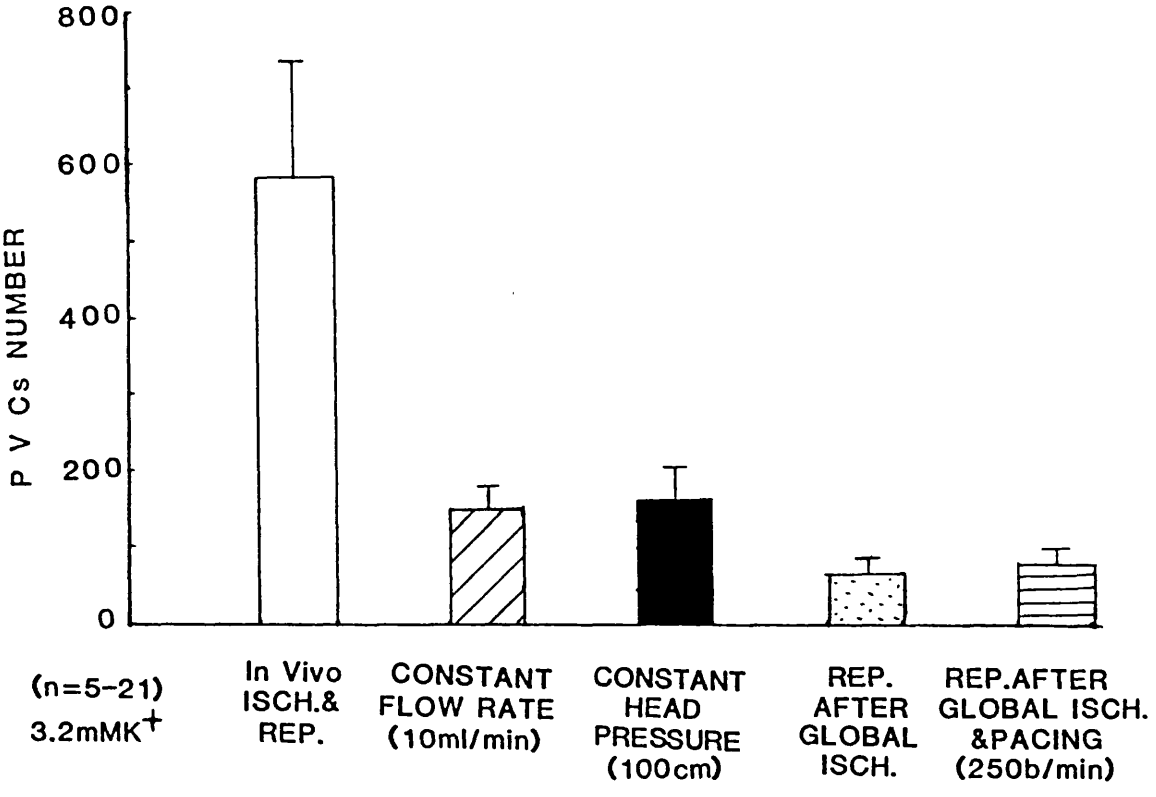


Fig. (16). Numbers of PVCs developed during reperfusion after: 10 minutes ischaemia *in vivo*, 10 minutes regional ischaemia using constant flow rate (10 $ml.min^{-1}$, 3.2 mM K^+), 10 min regional ischaemia under constant head pressure (100 cm H_2O, 3.2 mM K^+), 10 min global ischaemia (3.2 mM K^+) and 10 minutes global ischaemia and pacing (250 $b.min^{-1}$ started 2 min before the onset of ischaemia until the end of the experiment, 3.2 mM K^+).

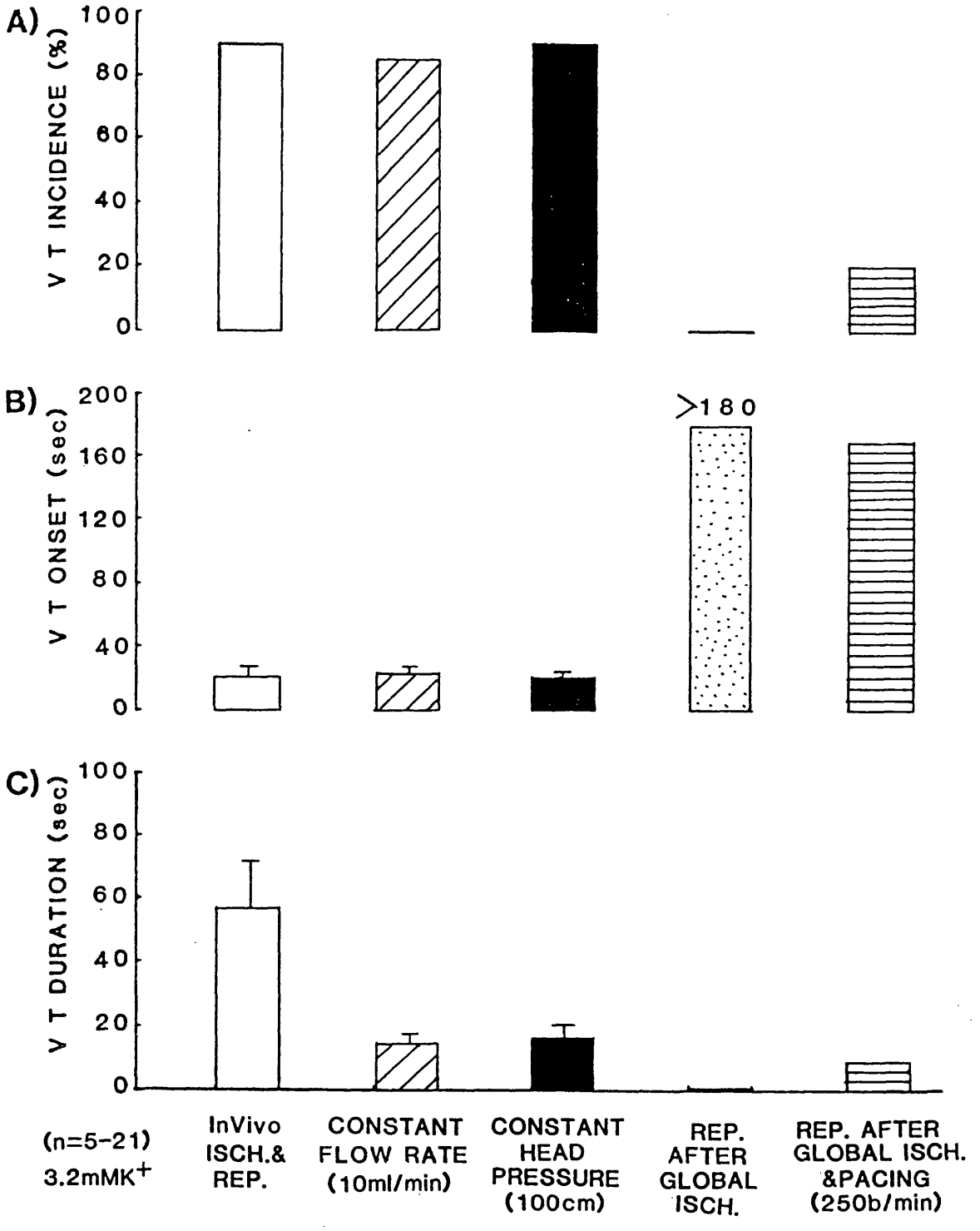


Fig. (17);a)Incidence, b) onset and c) duration of VT developed during reperfusion after: myocardial ischaemia *in vivo*, regional ischaemia using constant flow rate (10 ml.min^{-1}, 3.2 mM K^+), global ischaemia (3.2 mM K^+) and global ischaemia in the presence of pacing (250 b.min^{-1} started 2 min before the onset of ischaemia till the end of the experiment, 3.2 mM K^+).

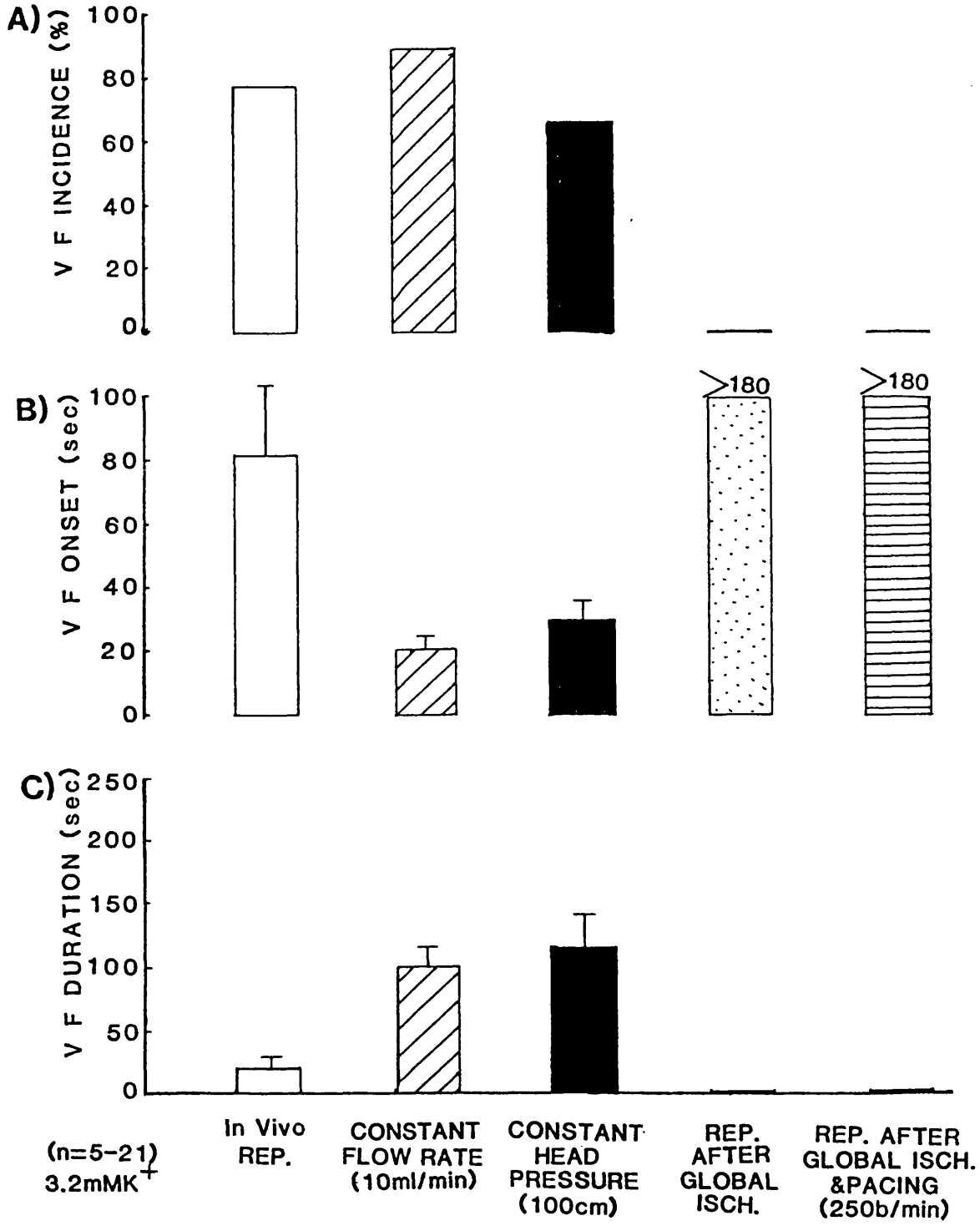


Fig. (18). a) incidence, b) onset and c) duration of VF developed during reperfusion after: myocardial ischaemia *in vivo*, regional ischaemia using constant flow rate (10 $ml.min^{-1}$, 3.2 mM K^+), regional ischaemia under constant head pressure (100 cm H_2O, 3.2 mM K^+) and global ischaemia in the presence of pacing (250 $b.min^{-1}$ started 2 min before the onset of ischaemia till the end of the experiment, 3.2 mM K^+).

models, while no VF developed on reperfusion after global ischaemia (n = 5). A longer VF onset and shorter VF duration were seen in the *in vivo* model than in the *in vitro* models (Figs. (18 b and c)

3.3: Relationship between head pressure and reperfusion induced arrhythmias

Preliminary experiments to study the effect of perfusion pressure on the development of reperfusion arrhythmias and coronary flow were performed. The results of these experiments are shown in Figures 19 - 22. From these experiments, it was found that PVCs number developed on reperfusion increases with increasing perfusion pressure until it becomes significantly different at a pressure 100 cm H_2O compared with 50 cm H_2O (Fig. (19b)). Similarly, the incidences of VT and VF were also found to increase with increasing perfusion pressure (Figs. (20a) and (21a)). The onset of VT and VF was reduced with high perfusion pressures while the duration of these arrhythmias increased at high perfusion pressures (Figures (20) and (21)). Figures (19a) and (22) show that there are significant positive correlations between constant head pressure (cm H_2O), and incidence of VT % ($r = 0.956$, $t = 4.584$, $Y = 0.89x + 21.76$), incidence of VF % ($r = 0.984$, $t = 7.903$, $Y = 0.69x + 52.7$) and coronary flow rate ($r = 0.997$, $t = 18.35$, $Y = 9.11x + 2.8$).

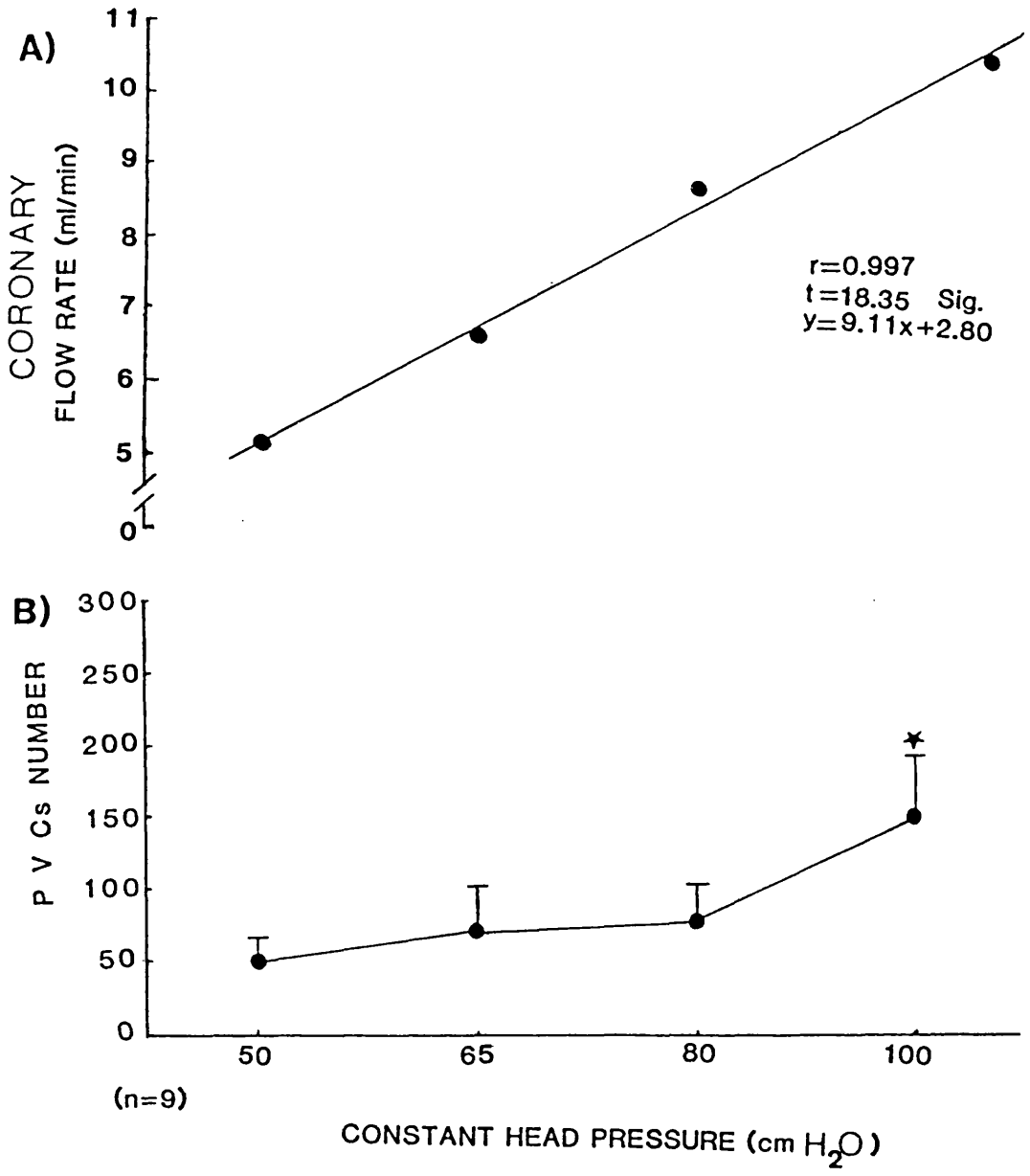


Fig. (19). A) Correlation between head pressure (cm H_2O) and coronary flow rate ($ml.min^{-1}$). B) Number of PVcs developed on reperfusion of regionally ischaemic myocardium under different head pressures (50 - 100 cm H_2O, 3.2 mM K^+) * $P < 0.05$

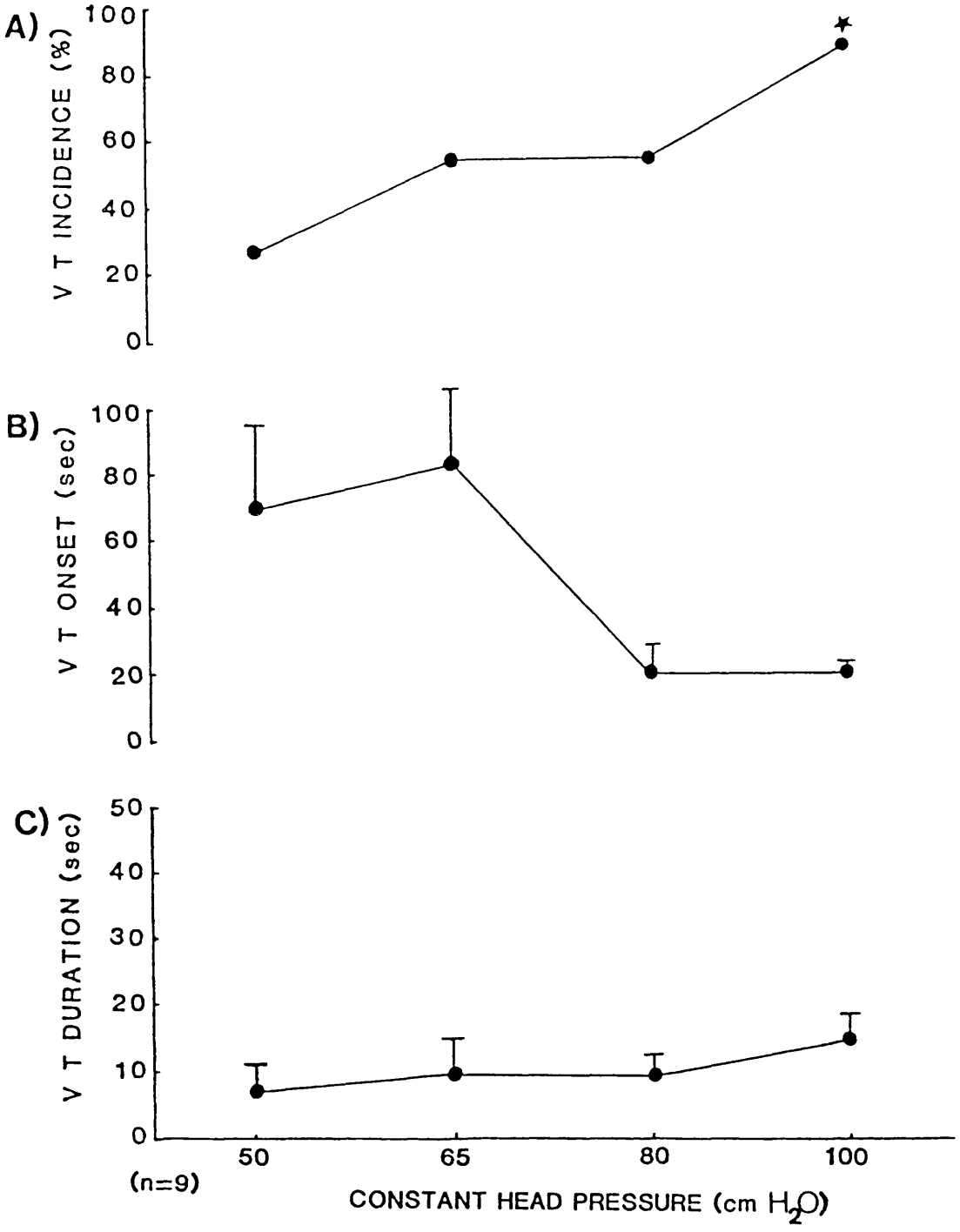


Fig. (20). a) Incidence, b)onset and c) duration of VT precipitated during reperfusion of regionally ischaemic myocardium under different head pressures (50 - 100 cm H_2O, 3.2 mM K^+).

* $P < 0.05$.

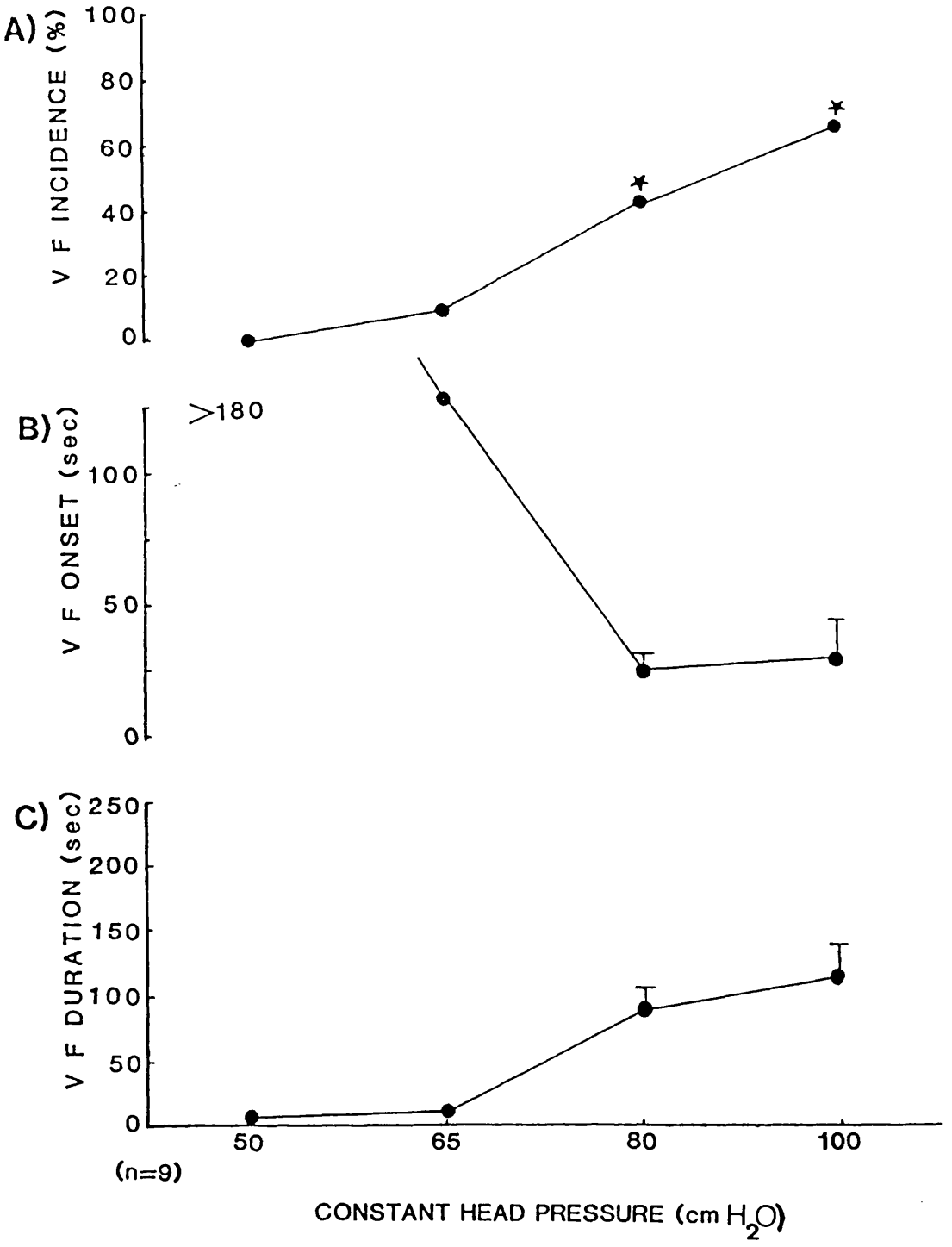


Fig. (21). a) Incidence, b) onset and c) duration of VF precipitated during reperfusion of regionally ischaemic myocardium under different head pressures (50 - 100 cm H_2O, 3.2 mM K^+).

* $P < 0.05$.

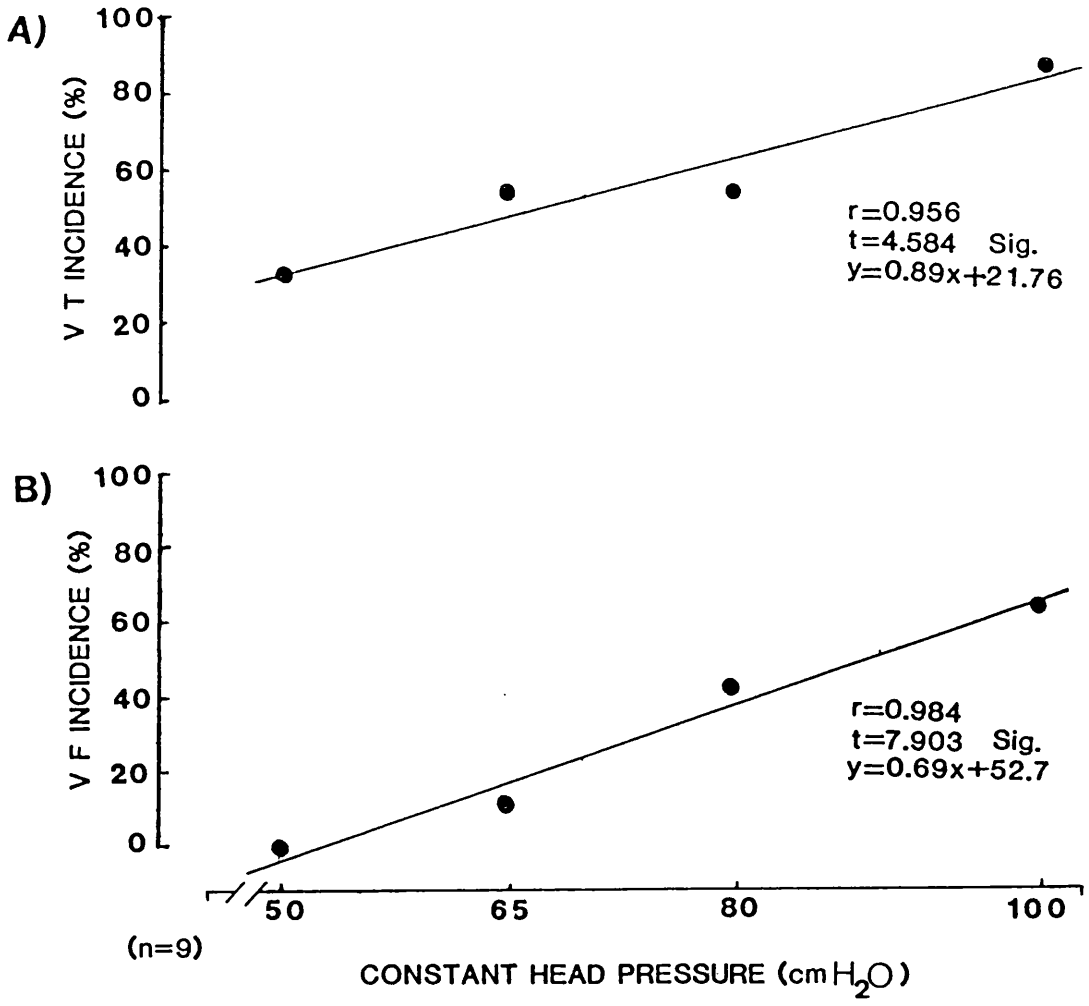


Fig. (22). Correlations between head pressure (cm H_2O) and A) VT incidence (%); B) VF incidence (%).

Section B: Discussion

Reperfusion induced arrhythmias in the isolated rat heart model used in the present study may have some resemblance to human arrhythmias which are thought to cause sudden cardiac death in man. This is supported by the absence of complete coronary occlusion in patients autopsied after sudden cardiac death suggesting that a lethal event may have followed reperfusion of ischaemic myocardium via spontaneous lysis of thrombus formed due to platelets aggregation, vasodilation of an obstructed artery or increased collateral flow to an ischaemic region(Corr and Witkowski, 1984). The development of ventricular arrhythmias during intracoronary thrombolysis in patients (Goldberg *et al.*, 1983) may provide also a support to the applicability of experimental reperfusion induced arrhythmias to the clinical situation. However, because it is difficult to obtain data on pharmacological prevention of VF and sudden death in man and higher primates because of the unpredictable onset of the arrhythmias, therapeutic attitudes will have to be based on data obtained from basic animal models. The aim should remain to induce VF using mechanisms which are closely associated with the development of VF in the clinical situation, e.g. regional ischaemia (Lubbe *et al.*, 1978) and reperfusion.

In the present study, the rat has been used as experimental animal. It has the following advantages: a) economic in price, b) surgery is easy, c) a small amount of drug is needed because of its small size, d) very little collateral flow exists in rat which is similar to healthy man, and e) myocardial ischaemia is

complete in rat due to very low collateral circulation which will produce high incidence of reperfusion induced arrhythmias which suggests that rat could be used reliably in antiarrhythmic evaluations (Bergey *et al.*, 1982). On the other hand,the rat has the following disadvantages when compared with other mammalian species: a) rat heart has different electrophysiology and electrocardiogram from other mammalian species. The rat heart has a briefer QRS duration and effective refractory period than dog heart for example. In addition, in the adult rat the T wave follows upon the QRS complex without an isoelectric ST segment, this is due to the brief duration and lack of a plateau phase of the rat ventricular myocardial action potential (Spear, 1981). b) The rat heart is small and is prone to spontaneous defibrillation particularly if a reentrant or circus mechanism is the cause of the arrhythmia because reentrant pathways may be relatively shorter than in other species with larger hearts. This could augment the antiarrhythmic activity of agents to be examined (Bergey *et al.*, 1982; Bergey and Beil, 1983). c) A marked seasonal variation in the incidence of ligation induced ventricular fibrillation has been shown in the rat which may be due to seasonal variations in hormonal, nervous and metabolic activity in the rat (Abrahamsson and Almgren, 1981). This is less of a problem *in vitro*.

In the present investigation, the *in vitro* model has been chosen because it has the following advantages over the *in vivo* model: a) Its simplicity. b) It gives as much information as the *in vivo* model. c) It is devoid of extracardiac reflexes and haemodynamic changes while coronary flow and heart rate can be

controlled. This means that any actions relating to arrhythmias are more likely to be the result of direct effects of the drug on the electrical activity of the heart and not due to non specific anti-ischaemic actions. d) The effect of the anaesthetic agents is avoided. e) The possibility of the effect of seasonal variation is also eliminated by the use of the *in vitro* model. f) There is a consistent occurrence and higher incidence of reperfusion induced arrhythmias in this model compared with the *in vivo* model. On the contrary, the *in vitro* model used in this study has the following disadvantages compared with the *in vivo* model: a) It ignores the effect of blood on reperfusion induced arrhythmias e.g. the role of free radicals generated by blood neutrophils and the effect of some prostanoid compounds synthetized by blood platelets on the severity of these arrhythmias. b) The need to use high flow rate in the *in vitro* model in order to achieve normal oxygen tension in the heart tissue because of the poor carrying capacity of the Kreb's solution compared with the high oxygen carrying capacity of blood haemoglobin. This high flow rate might affect the severity of reperfusion induced arrhythmias (Figures: (19) to(21)). c) It ignores the extracardiac reflexes which may indirectly affect the severity of the resulting arrhythmias. d) In this model, the heart is not working and there is no after load. This also can be expected to affect the severity of the arrhythmias.

In the present study, the *in vitro* model using a constant flow system has been used throughout the study. It has an advantage of the global ischaemia model in that it creates electrophysiological

and biochemical difference between the ischaemic and non ischaemic regions. This difference may have a marked effect on the development of reperfusion induced arrhythmias. The constant flow *in vitro* model has also an advantage over the constant pressure model in that the effect of coronary steal on reperfusion arrhythmias can be assessed. Furthermore, when the incidence of reperfusion induced VF was compared in the three *in vitro* models: global ischaemia, regional ischaemia using a constant head of pressure and regional ischaemia using a constant flow rate, it was found that the constant flow model had the highest incidence of ventricular fibrillation (Figs. (16) to (18)). On the other hand, this model has the drawback that the effect of the change in flow rate induced by vasodilators on reperfusion arrhythmias is not seen.

The highest incidence of reperfusion induced ventricular fibrillation was found to occur after 10 minutes coronary artery ligation in the constant flow *in vitro* model (Figs. (13) to (15)) this is consistent with what has been shown in dogs *in vivo* that susceptibility to VF during reperfusion was maximal after 10 minutes of ischaemia and the ventricular fibrillation threshold was greatly reduced on reperfusion after this period of myocardial ischaemia (Axelrod *et al.*, 1975; Corbalan *et al.*, 1976). In the anaesthetized rat model the optimal period of ischaemia required for the highest incidence of reperfusion induced arrhythmias was found to be 5 minutes (Kane *et al.*, 1984; Manning and Hearse, 1984), this could be due to the higher heart rate in this model than that in the *in vitro* model. On the other hand, in other studies using isolated guinea pig hearts and the anaesthetized dog,

reperfusion arrhythmias have been shown to be most frequent following 20 to 30 minutes of ischaemia (Balke *et al.*, 1981; Penny and Sheridan, 1983). This contradiction could be due to the variation in collateral circulation among the different species or due to the use of different models. In guinea pigs reperfusion was induced after global ischaemia (Penny and Sheridan, 1983) therefore longer periods of reduced flow perfusion could be required to produce myocardial ischaemia while the dog heart has been reported to have an extensive and variable degree of collateral blood supply (Bergey *et al.*, 1982) which may be responsible for the variation in the optimal period of ischaemia needed for the development of reperfusion induced arrhythmias among different studies using this species.

The present investigation shows similar incidence of reperfusion induced VT and VF in both the *in vivo* and the isolated rat heart models. This suggests that the severity of arrhythmias developing on reperfusion of ischaemic myocardium is not markedly affected by the extracardiac autonomic reflexes. The number of PVCs was higher in the *in vivo* model and this could be due to the fact that the heart rate is higher in the *in vivo* model than that in the *in vitro* model. Furthermore, the increase in the incidence of reperfusion arrhythmias as a result of increasing the rate of flow by increasing the head of pressure provides an evidence for the detrimental effect of sudden and rapid reperfusion of ischaemic myocardium. This concept is supported by the data of slow reperfusion (Chapter 4) in this study and by what had been demonstrated by Petropoulos and Meijne(1964) that gradual

rather than rapid reperfusion after reduced flow global ischaemia in the anaesthetized dog performed by perfusion of the circumflex branch of the left coronary artery with low-molecular dextran or Tyrode solution at low flow rate attenuated the associated ventricular arrhythmias. The data in this chapter acted as a preliminary work which guided the whole study in this thesis.

CHAPTER 4

"The effect of potassium, magnesium and calcium on reperfusion induced arrhythmias in the isolated rat heart"

Section A: Results

It was worthwhile examining the effects of K^+, Mg^{2+} and Ca^{2+} on reperfusion arrhythmias because these ions are known to affect the incidence of arrhythmias in man (Johnson *et al.*, 1979). Therefore a study of the effects of these ions on reperfusion induced arrhythmias was carried out.

4.1: Effect of potassium on reperfusion arrhythmias

There was no significant change in the number of PVCs developed on reperfusion of ischaemic myocardium with different potassium concentrations (Fig. (23)). Potassium (2.5 - 10.0 mM) produced a concentration dependent reduction in the incidence of VT and VF (Figs. (24a) and (25a)). There was no significant effect of potassium on the onset of VT and VF or on the duration of VT (Fig. (24b and c) and Fig. (25b)). The duration of VF was negatively correlated with the potassium concentration ($r = -0.913$). Over the range of 2.5 - 10.0 mM there was a significant negative correlation between potassium concentration and the incidence of VF ($r = -0.991$) (Fig. (26a)).

4.2: Haemodynamic effects of changing potassium

Potassium was found to cause a concentration dependent vasodilation as indicated by a reduction in perfusion pressure (Fig. (27a)) and positive inotropic effect (Fig. (27b)). In Fig. (26b) it can be seen that there was a significant negative correlation

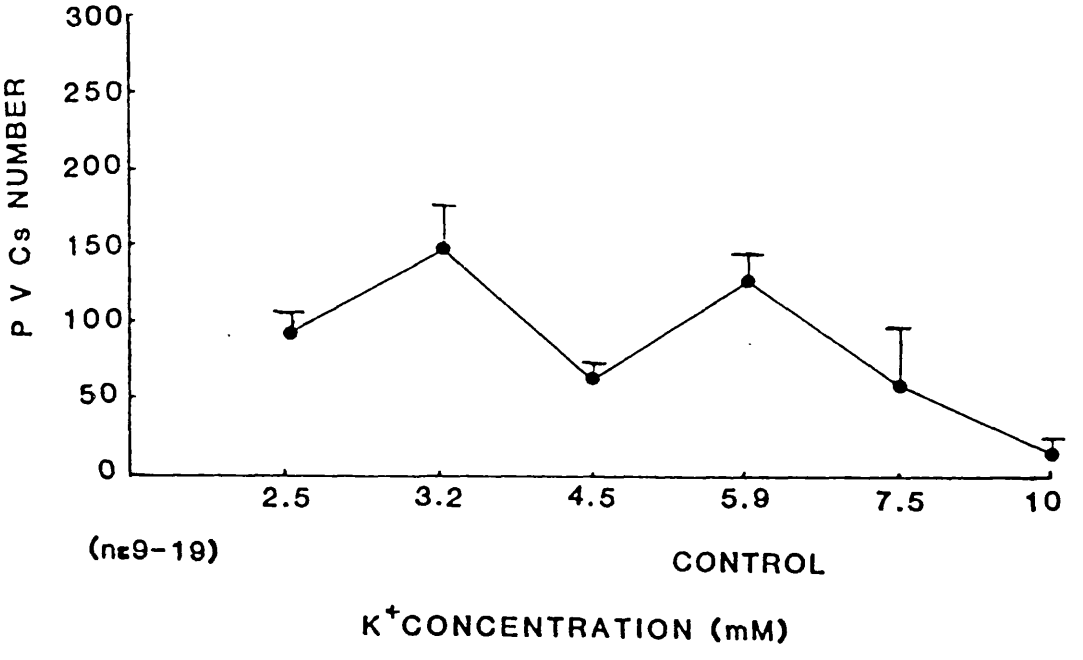


Fig. (23). Effect of potassium on the number of PVCs developed during reperfusion in the isolated rat heart. Magnesium 1.2 mM; calcium 1.2 mM.

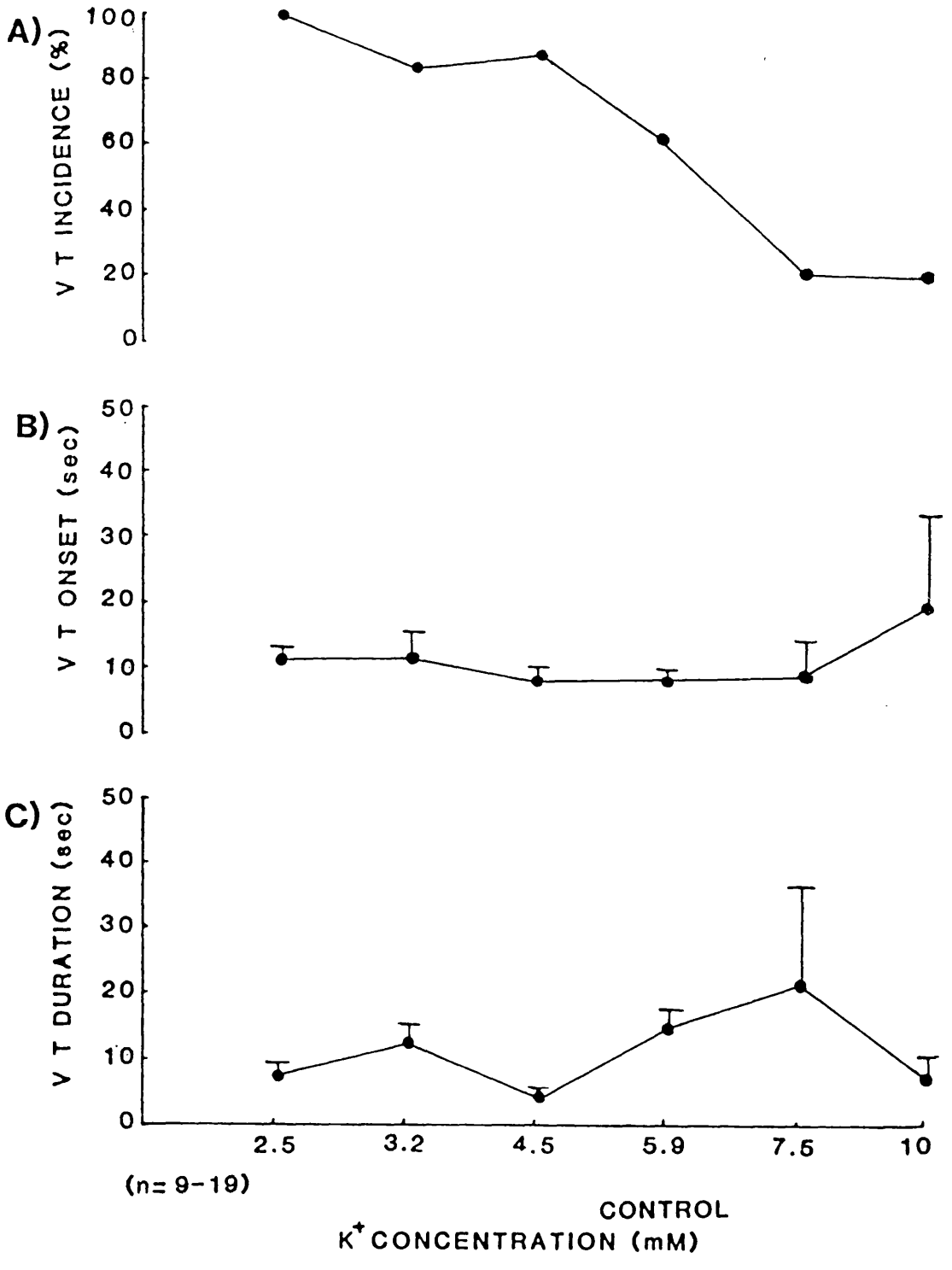


Fig. (24). Effects of potassium on a) the incidence, b) onset and c) duration of VT developed during reperfusion in the isolated rat heart. Magnesium 1.2 mM; calcium 1.2 mM. Values in B and C in this and other Figures are means ± S.E.M.

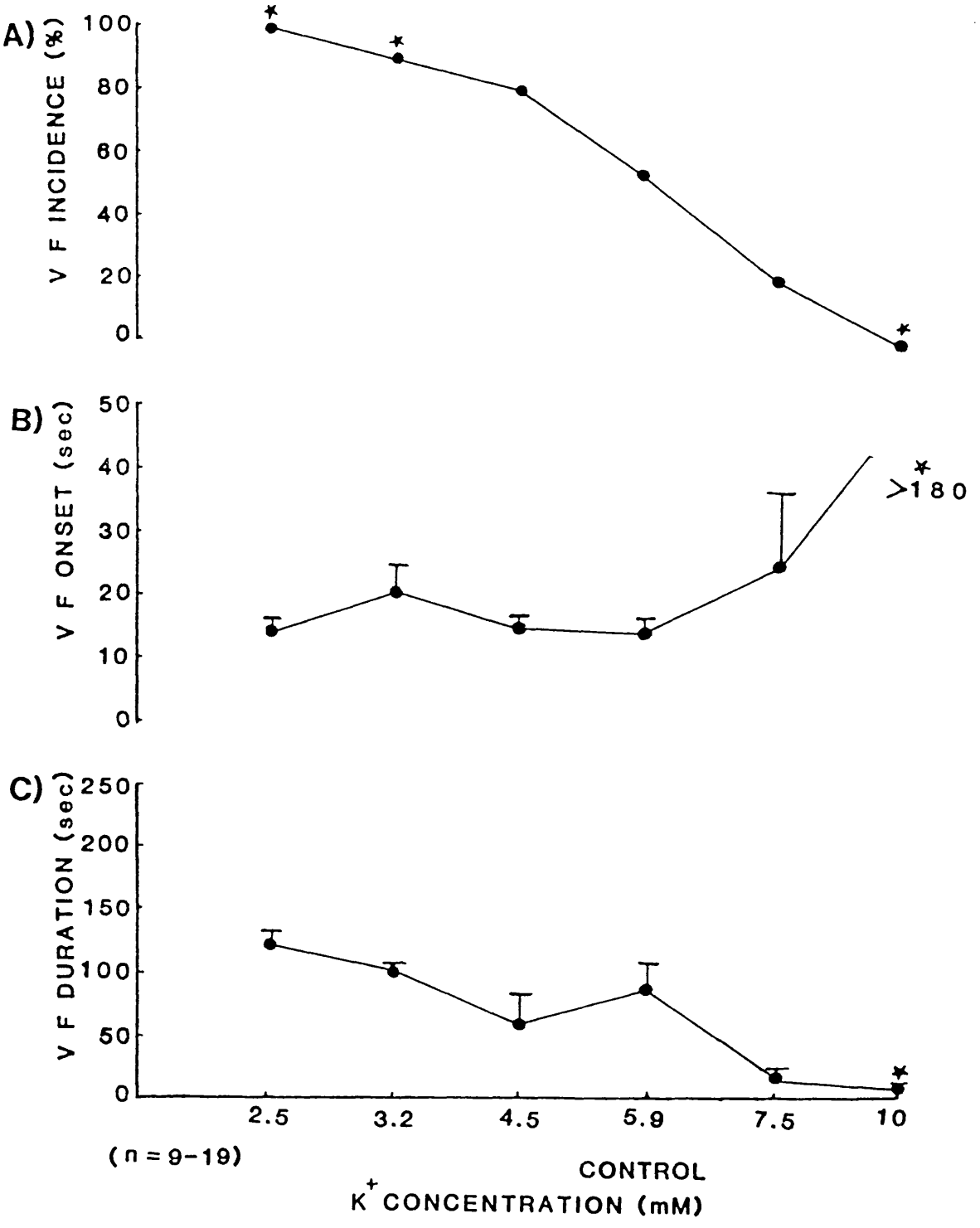


Fig. (25). Effects of potassium on a) the incidence, b) onset and c) duration of VF developed during reperfusion in the isolated rat heart. Magnesium 1.2 mM; calcium 1.2 mM. In this and other Figures * $P < 0.05$. C.W. potassium 5.9 mM; magnesium 1.2 mM; calcium 1.2 mM.

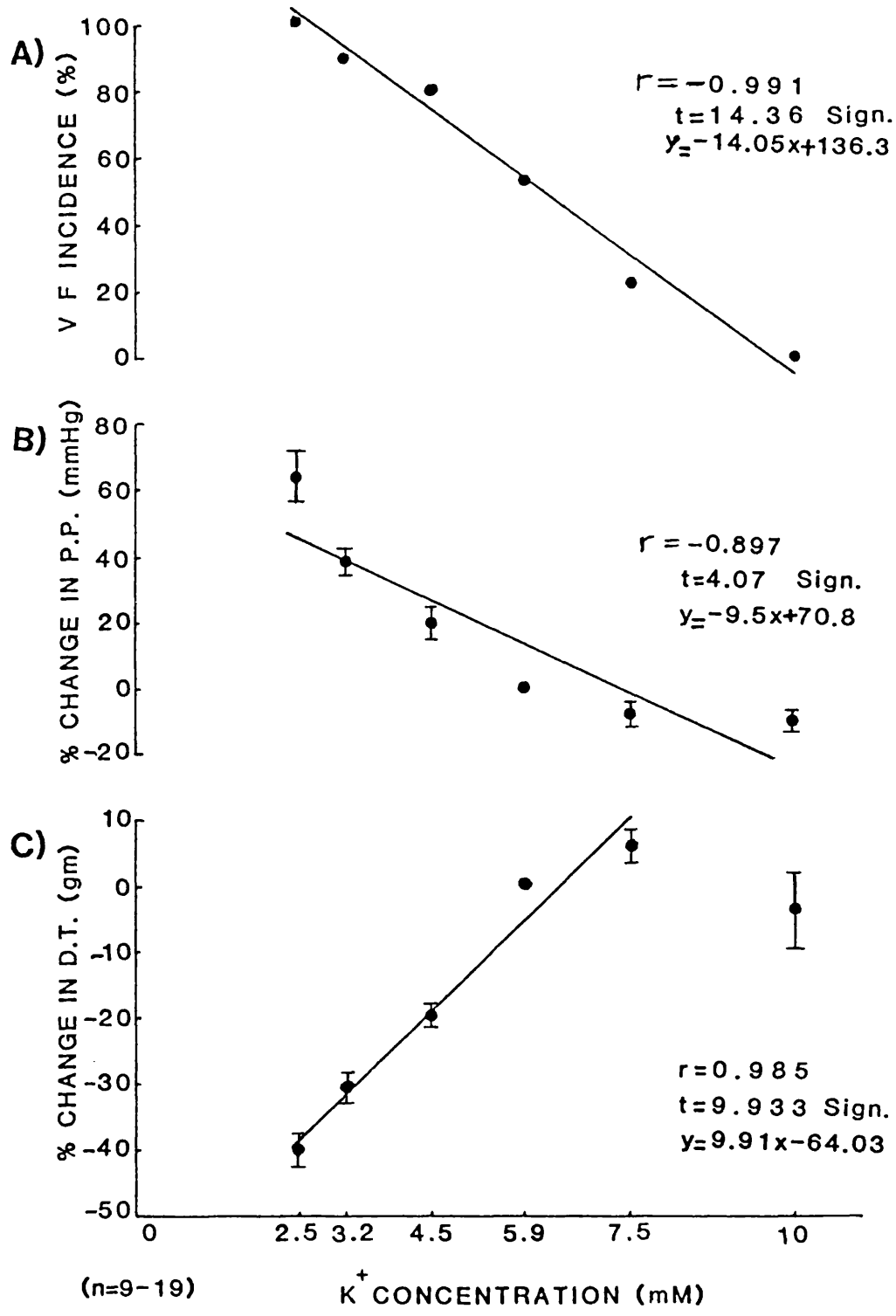


Fig. (26). Correlation between potassium concentration and a) VF incidence %, b) change in perfusion pressure % and c) change in developed tension % in the isolated rat heart. Magnesium 1.2 mM; calcium 1.2 mM.

between potassium concentration and change in perfusion pressure. The change in developed tension was positively correlated with the potassium concentration (Fig. (26c)).

4.3: Effect of magnesium on reperfusion arrhythmias

Magnesium at a concentration 4.8 mM reduced the number of PVCs when compared with lower concentrations (Fig. (28)). Magnesium (0 - 4.8 mM) also produced a concentration dependent reduction in the incidence of VT ($r = -0.976$) and VF ($r = -0.979$) (Figs. (29a), (30a) and (31a)). Magnesium at concentrations 2.4 and 4.8 mM significantly increased the onset and reduced the duration of VT (Fig. (29b and c))and VF (Fig. (30b and c)).

4.4: Haemodynamic effects of changing magnesium

Magnesium (0 - 4.8 mM) produced a concentration dependent bradycardia (Fig. (31b) and Fig. (32c)), vasodilation (as indicated by the fall in perfusion pressure, Fig. (32a)) and negative inotropic action (Fig. (31c) and Fig. (32b)).

4.5: Effect of magnesium and potassium on reperfusion arrhythmias

Tables (1 and 2) show the effects of a wider range of combinations of magnesium and potassium on the development of VF and

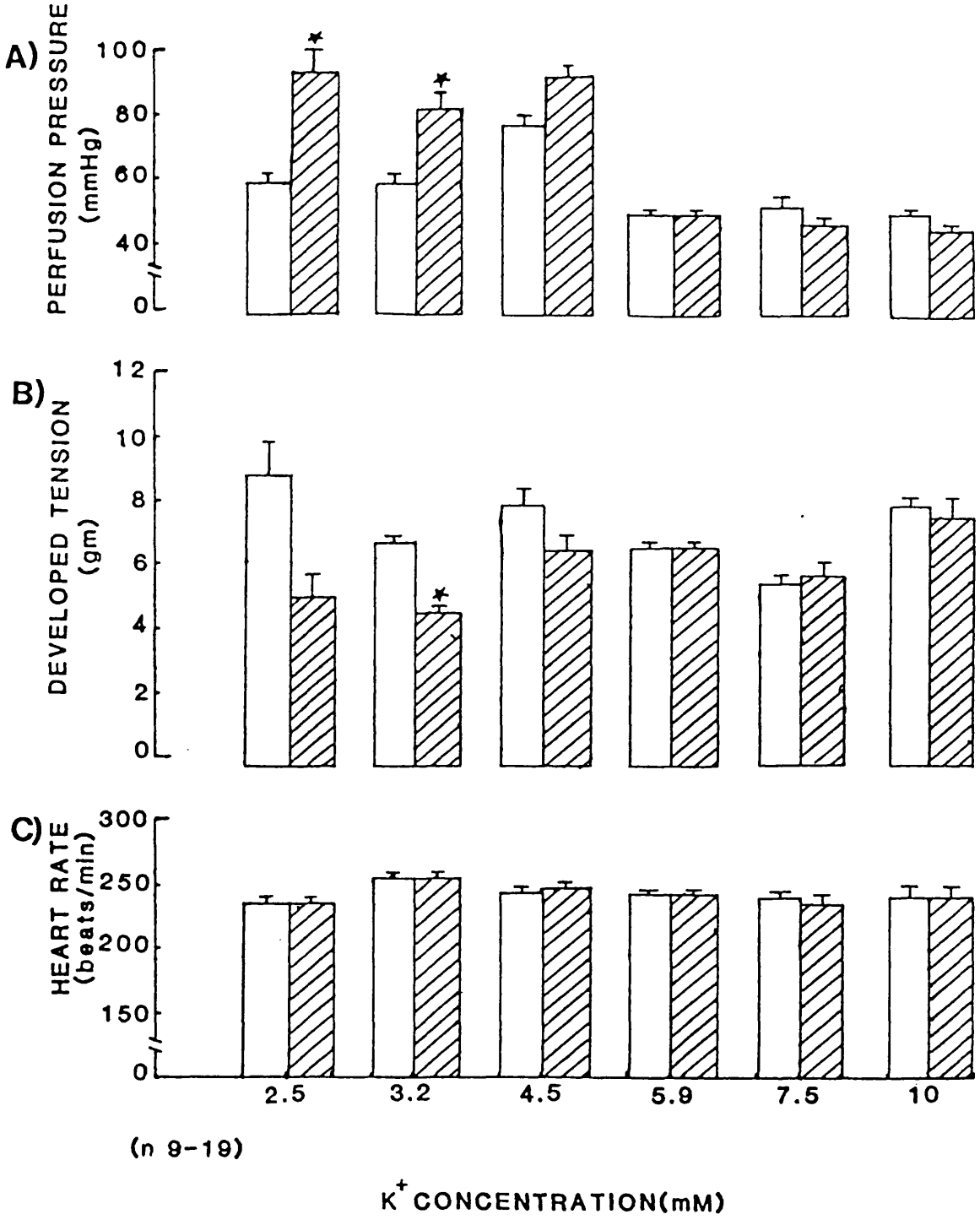


Fig. (27). Effects of potassium on a) perfusion pressure, b) developed tension & c) heart rate in the isolated rat heart. Magnesium 1.2 mM; calcium 1.2 mM. In this Figure and other Figures open columns represent 5.9 mM K^+ and hatched columns represent changed K^+ concentration (open columns are control for hatched columns).

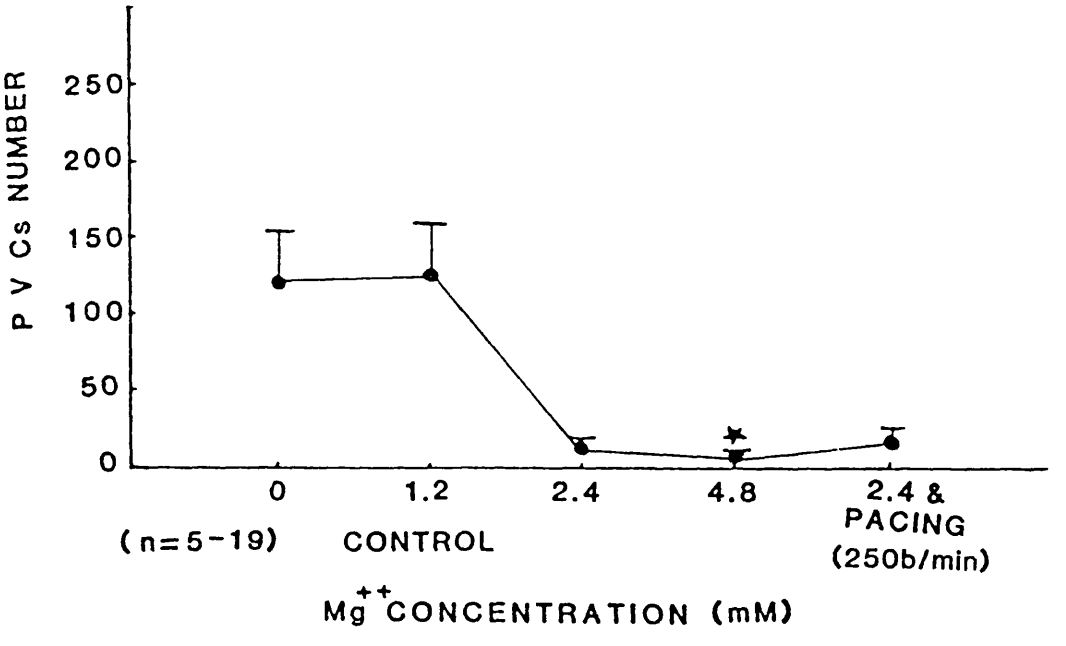


Fig. (28). Effect of magnesium on the number of PVCs developed during reperfusion in the isolated rat heart. Potassium 5.9 mM; calcium 1.2 mM.

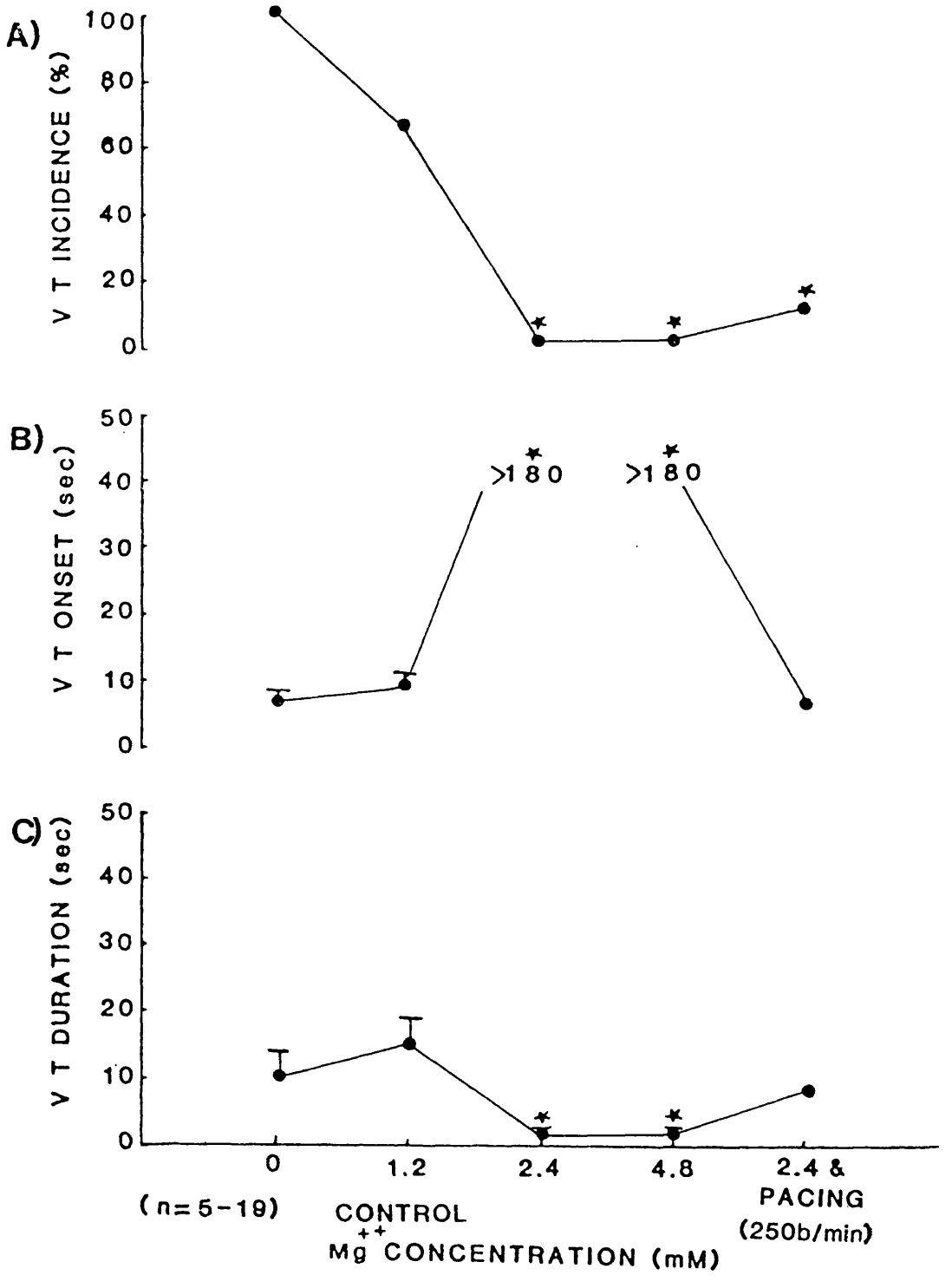


Figure (29). Effects of magnesium on a) the incidence, b) onset and c) duration of VT developed during reperfusion in the isolated rat heart. Potassium 5.9 mM; calcium 1.2 mM.

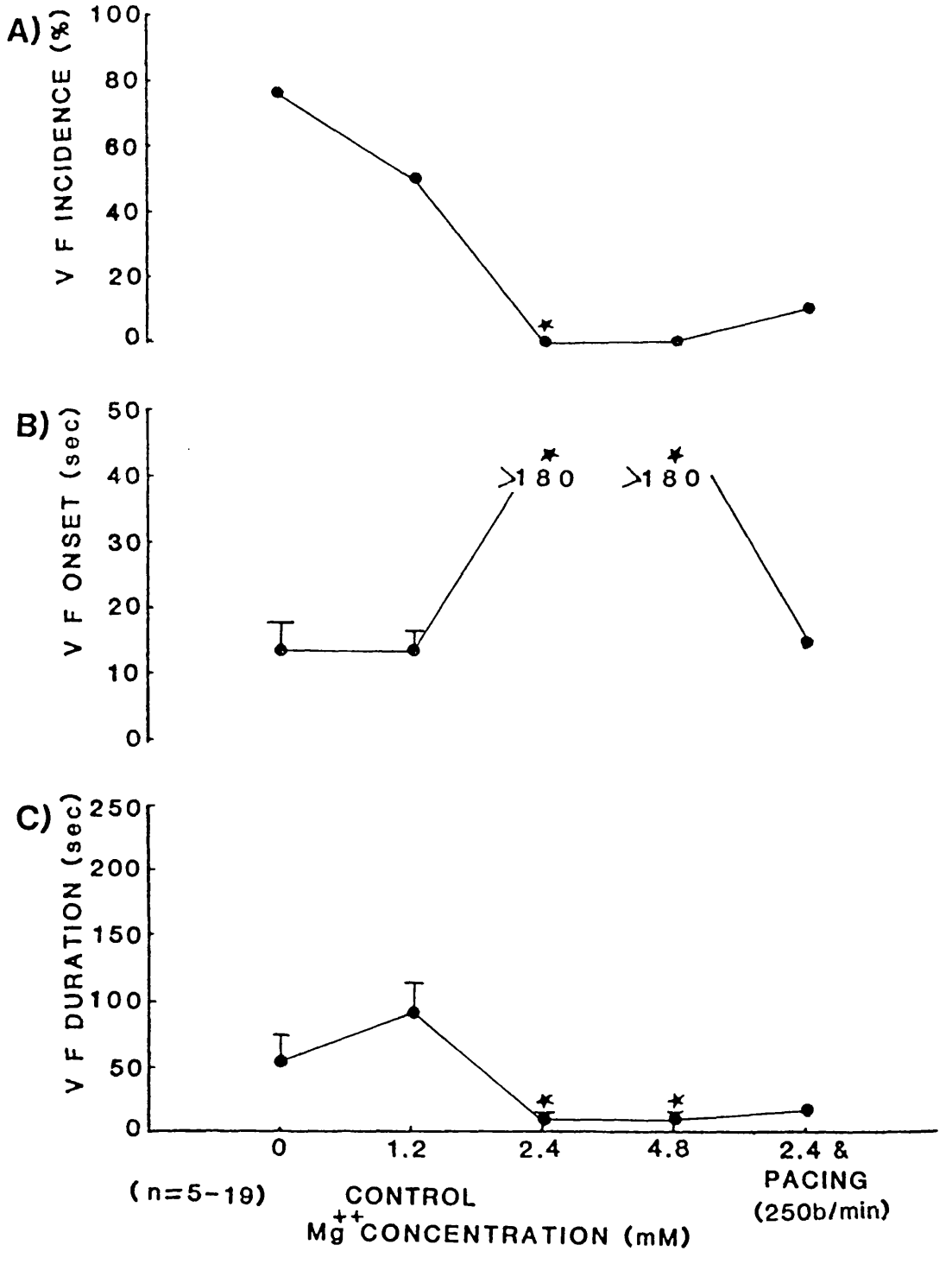


Fig. (30). Effects of magnesium on a) the incidence, b) onset and c) duration of VF developed during reperfusion in the isolated rat heart. Potassium 5.9 mM; calcium 1.2 mM.

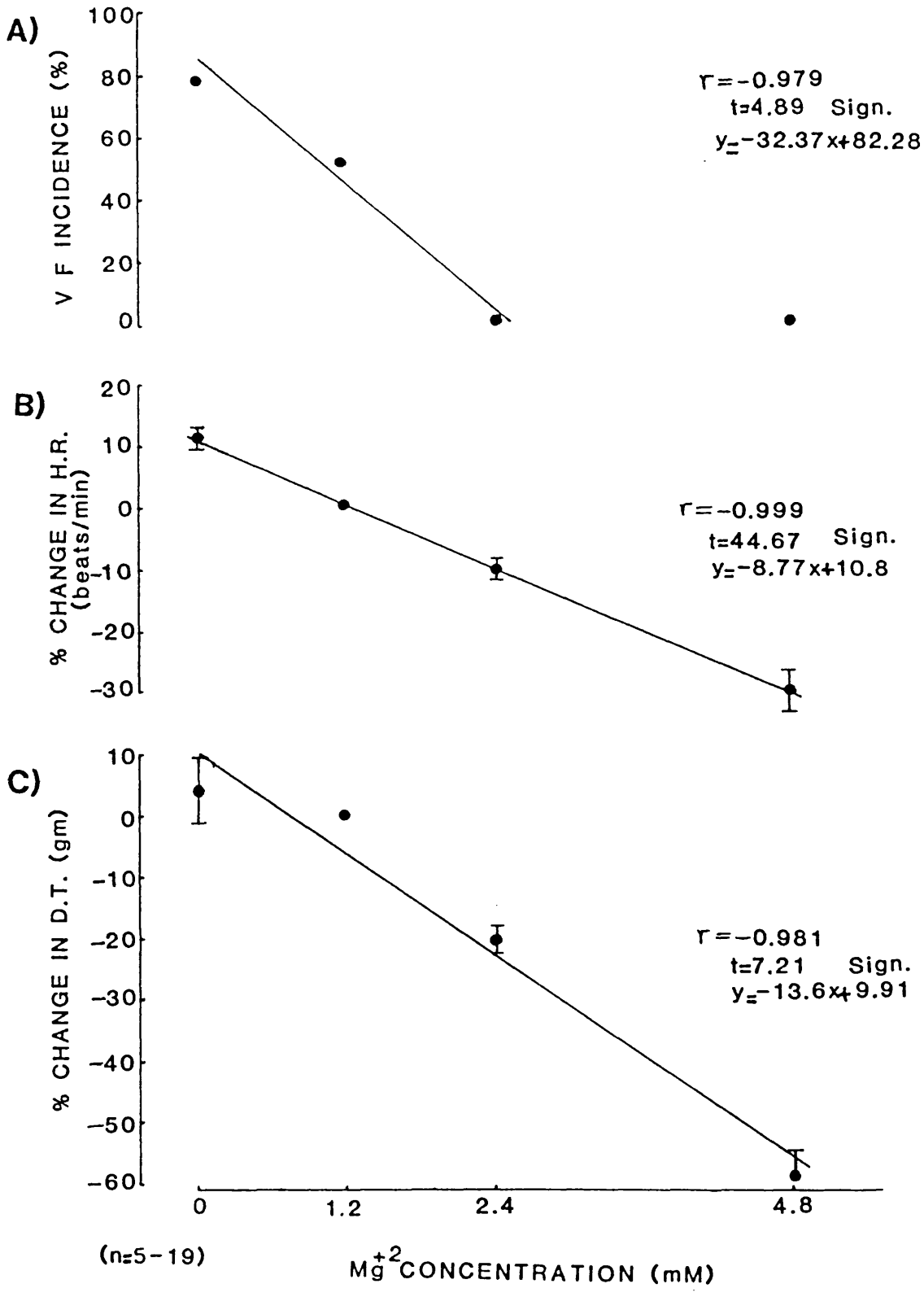


Fig. (31). Correlation between magnesium concentration and a) VF incidence %, b) change in heart rate % and C) change in developed tension % in the isolated rat heart. Potassium 5.9 mM; calcium 1.2. mM.

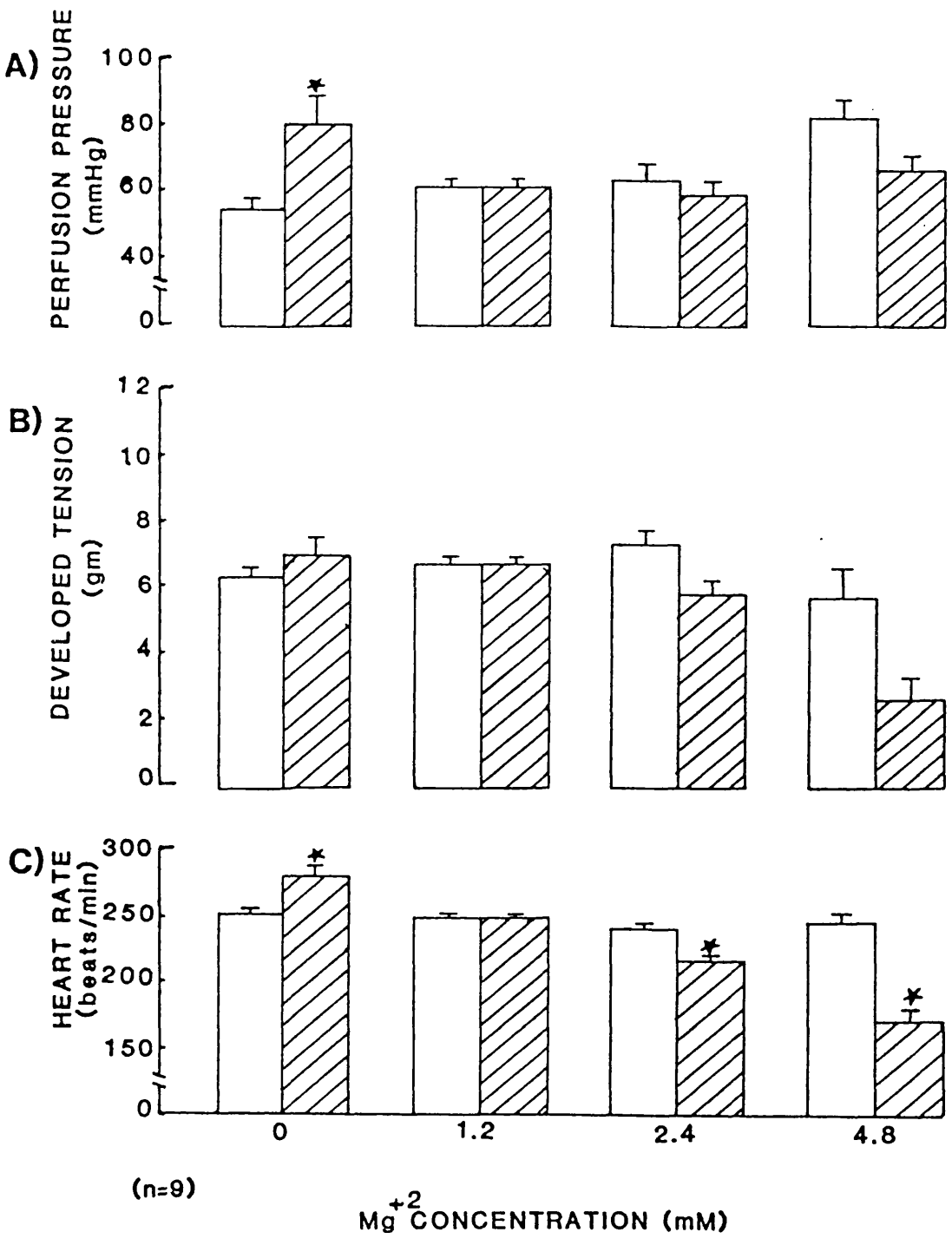


Fig. (32). Effects of magnesium on a) perfusion pressure, b) developed tension and c) heart rate in the isolated rat heart. Potassium 5.9 mM; calcium 1.2 mM.

VT. The combination of low magnesium and low potassium exerted a potent arrhythmogenic effect while increasing the concentration of one or both of these ions reduced the incidence of these serious arrhythmias. There was a significant negative correlation between the total magnesium plus potassium concentration (mM) and the incidence of VF ($r = -0.916$) and VT ($r = -0.874$). These results demonstrate the additive antiarrhythmic action of magnesium and potassium over the concentration ranges studied. There was no significant correlation between the total magnesium plus potassium concentration (mM) and the onset and duration of VT (Tables 3 and 4) and VF (Tables 5 and 6).

4.6: Effect of electrical pacing on the antiarrhythmic action of magnesium

In order to determine the effect of the magnesium induced bradycardia (Fig. (32c)) on reperfusion arrhythmias, a series of experiments were carried out in which heart rate was maintained constant at 300 b.p.m. by means of electrical pacing. The results of these experiments are shown in Table (7b). The low potassium concentration (3.2 mM) used in these experiments results in a high incidence of arrhythmias at the normal magnesium concentration of 1.2 mM (Fig. (25a)). Despite this and the fact that heart rate was maintained at 300 b.p.m. it can be seen that magnesium was still capable of reducing the development of VF in a concentration dependent manner (Table (7b)). However, when compared with hearts which were not paced (Table (7a)) it is clear that the protective effect of magnesium is reduced in the paced hearts as there is

Table 1. Effects of different combinations of Mg^{2+} and K^+ on the % incidence of reperfusion induced VT in the isolated rat heart. Calcium 1.2 mM (n = 5 - 21).

Mg^{++}(mM) \ K^+(mM)	2.5	3.2	4.5	5.9	7.5	10.0
0			80	100	44	
1.2	100	86	90	63	22	22
2.4		36	60	31	33	
4.8		22	20	0		

Table 2. Effects of different combinations of Mg^{2+} and K^+ on the % incidence of reperfusion induced VF in the isolated rat heart. Calcium 1.2 mM (n = 5 - 21)

Mg^{++}(mM) \ K^+(mM)	2.5	3.2	4.5	5.9	7.5	10.0
0			100	78	44	
1.2	100	90	80	53	22	0
2.4		36	60	23	33	
4.8		11	20	0		

Table 3. Effects of different combinations of Mg^{2+} and K^{+} on the onset (sec) of reperfusion induced VT in the isolated rat heart. Calcium 1.2 mM (n = 5 - 21)

Mg^{2+}(mM) \ K^{+}(mM)	2.5	3.2	4.5	5.9	7.5	10
0			4.7 ± 0.4	6.0 ± 1.2	3.9 ± 0.4	
1.2	10.2 ± 2.3	12.4 ± 4.4	9.4 ± 1.9	8.1 ± 2.1	9.5 ± 5.5	19.3 ± 15.3
2.4		14.2 ± 6.3	6.7 ± 2.3	24.0 ± 17.0	7.7 ± 2.2	
4.8		23.0 ± 12.5	8.0	>180 ± 0		

Table 4. Effects of different combinations of Mg^{2+} and K^{+} on the duration (sec) of reperfusion induced VT in the isolated rat heart. Calcium 1.2 mM (n = 5 - 21)

Mg^{2+}(mM) \ K^{+}(mM)	2.5	3.2	4.5	5.9	7.5	10
0			10.2 ± 3.3	10.7 ± 3.3	7.4 ± 1.8	
1.2	7.8 ± 1.2	12.9 ± 2.8	4.8 ± 0.7	15.3 ± 3.3	22.7 ± 13.8	7.2 ± 3.2
2.4		6.8 ± 1.8	13.3 ± 5.8	39.7 ± 22.4	12.0 ± 1.6	
4.8		2.7 ± 2.2	8.0	o ± o		

Table 5. Effects of different combinations of Mg^{2+} and K^{+} on the onset (sec) of reperfusion induced VF in the isolated rat heart. Calcium 1.2 mM (n = 5 - 21)

Mg^{2+}(mM) \ K^{+}(mM)	2.5	3.2	4.5	5.9	7.5	10
0			9.5 ± 2.5	13.7 ± 4.2	11.4 ± 2.3	
1.2	14.5 ± 1.5	20.6 ± 4.7	14.9 ± 2.2	13.6 ± 2.7	25.0 ± 12.0	> 180 ± 0
2.4		12.9 ± 3.9	14.5 ± 1.8	13.8 ± 1.8	19.7 ± 1.6	
4.8		o	17	> 180 ± 0		

Table 6. Effects of different combinations of Mg^{2+} and K^{+} on the duration (sec) of reperfusion induced VF in the isolated rat heart. Calcium 1.2 mM (n = 5 - 21)

Mg^{2+}(mM) \ K^{+}(mM)	2.5	3.2	4.5	5.9	7.5	10
0			114.4 ± 36.7	51.7 ± 23.1	90.7 ± 26.9	
1.2	125.8 ± 21.3	99.0 ± 16.1	58.2 ± 24.8	87.7 ± 22.0	12.5 ± 5.5	0 ± 0
2.4		106 ± 40.3	30.3 ± 29.1	14.0 ± 9.6	78.0 ± 43.5	
4.8		180	11.5	0 ± 0		

Table 7. Effects of electrical pacing (300 b.p.m.) on the antiarrhythmic action of magnesium. Magnesium was changed ten minutes before coronary ligation. Coronary flow 10 $ml.min^{-1}$ in A and B. In 7C hearts were perfused at a constant head of pressure (100 cm H_2O). Potassium 3.2 mM, calcium 1.2 mM. * $p < 0.05$ c.w. magnesium 1.2 mM

	Mg^{++} mM	n	PVC's/ 3 min	% Incid.	VT Onset sec	Dur. sec	% incid.	VF Onset sec	Dur (sec)
A) Unpaced Flow 10$ml.min^{-1}$	1.2	21	142±34	86	12±4	13±3	90	21±5	99±16
	2.4	14	37±14*	36*	14±6	7±2	36*	13±4	106±4
	4.8	9	20±9*	22*	23±12	3±2	11*	<1	180
B) Paced 300 b.p.m. Flow 10$ml.min^{-1}$	1.2	9	97±35	78	8±2	11±4	100	18±3	102±27
	2.4	9	127±29	89	17±5	16±4	78	31±6	149±6
	4.8	9	60±13	78	39±21	7±1	56*	27±8	153±8
C) Paced 300 b.p.m. constant pressure (100 cm H_2O)	1.2	18	145±28	94	21±5	17±3	83	25±4	149±5
	2.4	9	83±22	78	14±4	13±3	67	27±3	127±22
	4.8	9	158±28	89	23±3	15±3	33*	40±2	139±2

no significant reduction in the incidence of VT or in the number of PVCs.

4.7: Effect of magnesium on reperfusion arrhythmias in hearts perfused at a constant head of pressure and constant heart rate

Under the constant flow conditions of the above experiments, the fall in perfusion pressure induced by the vasodilator action of magnesium could influence the distribution of coronary flow and the rate of reperfusion of the ischaemic tissue via a coronary steal effect. Slow reperfusion has been shown to reduce the severity of reperfusion induced arrhythmias (Chapter 1.7 and Figs. (33), (34) and (35)), therefore a series of experiments were carried out in which hearts were perfused at a constant head of pressure. Under these conditions coronary flow can vary and is determined by coronary resistance. These hearts were also paced at 300 b.p.m. in order to prevent magnesium induced bradycardia. The results of these experiments are shown in Table (7c). Magnesium produced a concentration dependent reduction in VF, however none of the other arrhythmia parameters were affected by magnesium. Therefore, these results are very similar to those shown in Table (7b) where coronary flow was not altered.

4.8: Effects of post-ligation administration of potassium and magnesium

The effects of 10 mM potassium and 4.8 mM magnesium have been studied when administered just before reperfusion. These late

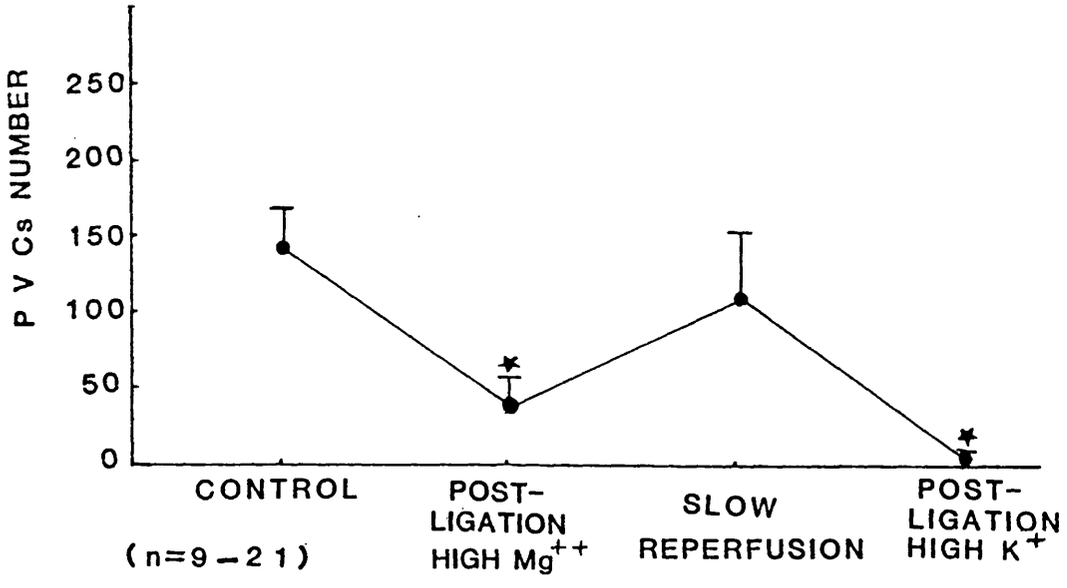


Fig. (33). Effects of post-ligation increase in Mg^{2+}; slow reperfusion, and post-ligation increase in K^{+} on the number of PVCs developed during reperfusion. Potassium has been increased from 3.2 mM to 10 mM; magnesium from 1.2 mM to 4.8 mM.

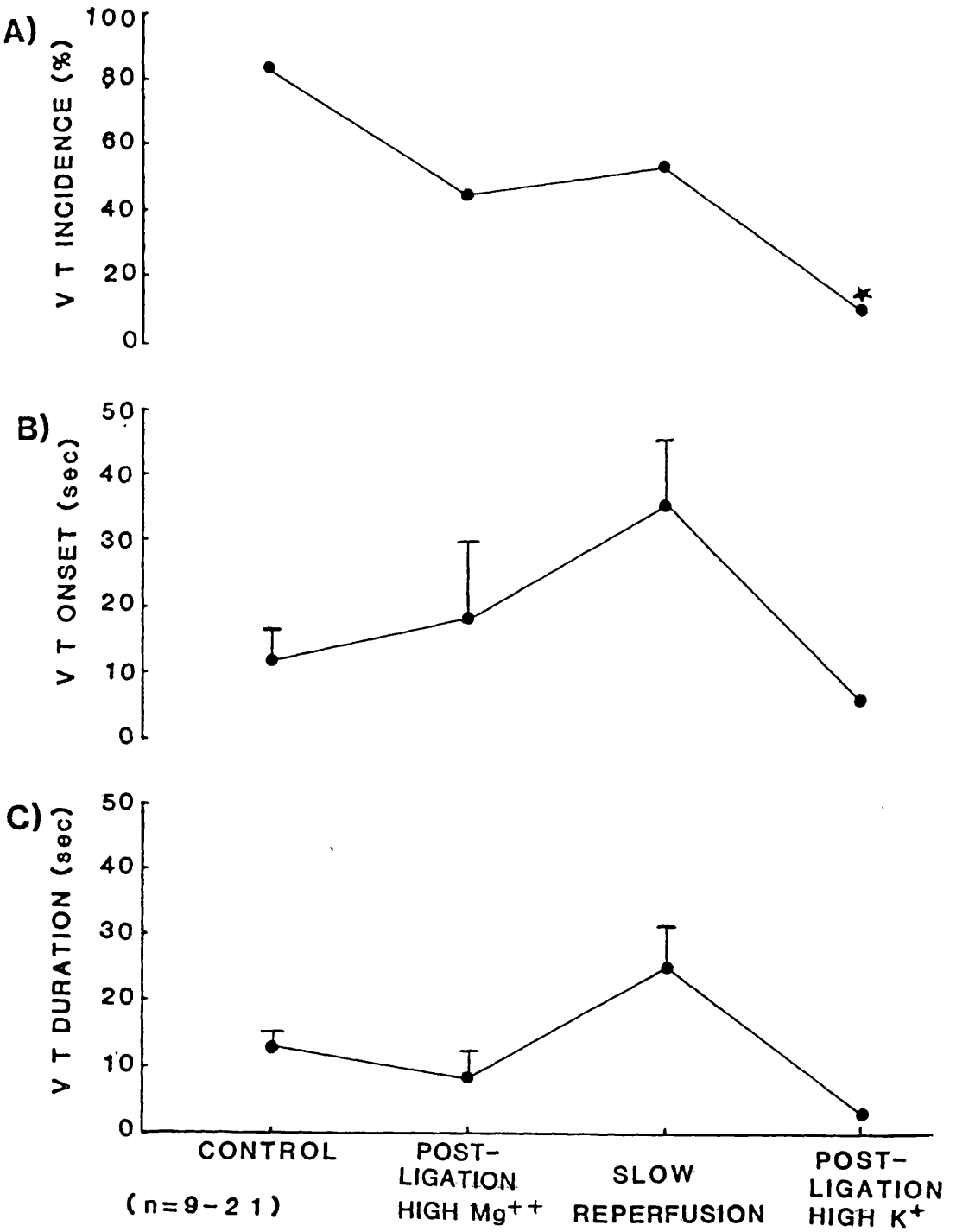


Fig. (34). Effects of post-ligation increase in Mg^{2+}, slow reperfusion and post-ligation increase in K^{+} on a) the incidence, b) onset and c) duration of VT developed during reperfusion. Potassium has been increased from 3.2 mM to 10 mM; magnesium from 1.2 mM to 4.8 mM.

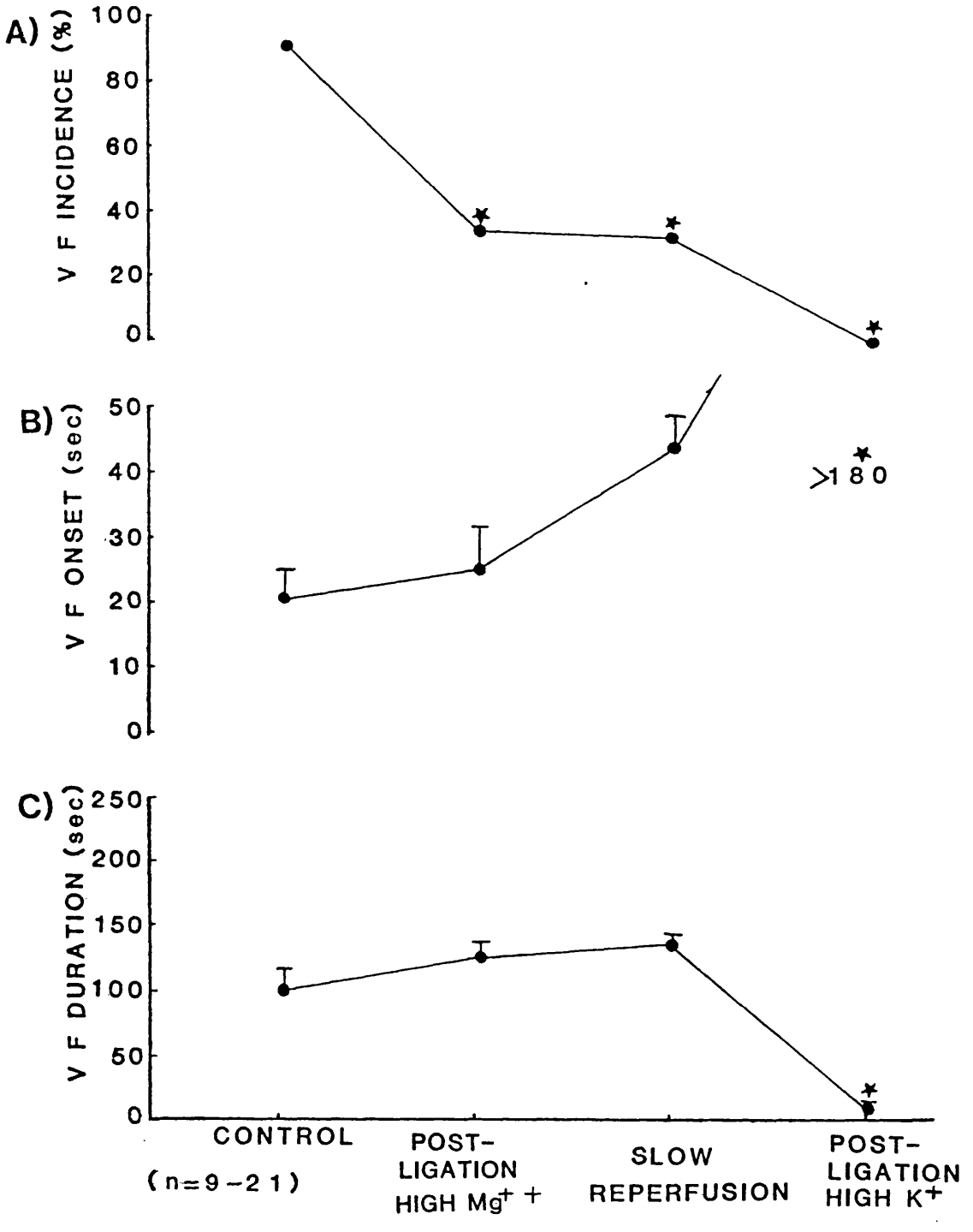


Fig. (35). Effects of post-ligation increase in Mg^{2+}, slow reperfusion and post-ligation increase in K^{+} on a) the incidence, b) onset and c) duration of VF developed during reperfusion. Potassium has been increased from 3.2 mM to 10 mM; magnesium from 1.2 mM to 4.8 mM.

changes in ionic composition would reduce any anti-ischaemic action of these ions. To allow sufficient time for complete mixing to occur in the dead space of the perfusion system the ionic changes were made in groups of nine hearts 2 min before reperfusion i.e. 8 min after coronary ligation. In these experiments the control group of animals (n = 21) had potassium and magnesium concentrations of 3.2 mM and 1.2 mM respectively in order to induce a high incidence of arrhythmias.

Reperfusion in the presence of a post ligation increase in magnesium (4.8 mM) reduced the number of PVCs (Fig. (33)) and the incidence of VT (Fig. (34a)) and VF (Fig. (35a)). The reduction in VF incidence and PVCs number were significant. There was no significant change in the onset and duration of VT (Fig. (34b and c)) and VF (Fig. (35b and c)). Therefore magnesium still had an antiarrhythmic action when given post ligation, however its beneficial effect was less than when it was given before ligation (Figs. (28), (29) and (30)).

The post ligation administration of potassium (10 mM) completely prevented the development of VF (Fig. (35)) and reduced the incidence of VT (Fig. (34a)) and the number of PVCs (Fig. (33)). There was no significant change in the onset and duration of VT (Fig. (34 b and c)). Therefore, the protective effect of potassium (10 mM) was the same whether it was given before or after coronary ligation.

4.9: Effect of calcium on reperfusion arrhythmias

Unlike potassium and magnesium, calcium had an adverse effect on the development of reperfusion induced arrhythmias. Increasing the calcium concentration (0.6 - 2.4 mM) produced no significant effect on the number of PVCs (Fig. (36)), increased the incidence of VT (Fig. (37a))and VF (Fig. (38a)) and also prolonged the duration of VF (Fig. (38c)). At 0.6 mM calcium the incidence of VT was significantly reduced when compared with calcium concentrations of 1.2 or 2.4 mM. There was no significant change in the onset of VT (Fig. (37b)) and VF (Fig. (38b)) with changing calcium concentration.

4.10: Haemodynamic effects of changing of calcium

Calcium (0.6 - 2.4 mM) produced concentration dependent increases in developed tension (Fig. (39b)) and heart rate (Fig. (39c)), and a fall in perfusion pressure (Fig. (39a)). There was a significant positive correlation between calcium concentration and change in heart rate % (Fig. (40b)).

4.11: Effect of verapamil on reperfusion arrhythmias

Verapamil, the widely used calcium channel antagonist, at concentrations 10^{-8} and 10^{-7} M produced changes in the development of reperfusion arrhythmias similar to those produced by decreasing the concentration of calcium. Verapamil (10^{-8} and 10^{-7} M) produced concentration dependent reduction in the incidence of VT (Fig. (42a)) and VF (Fig. (43a)). The time of onset (Fig. (43b)) and

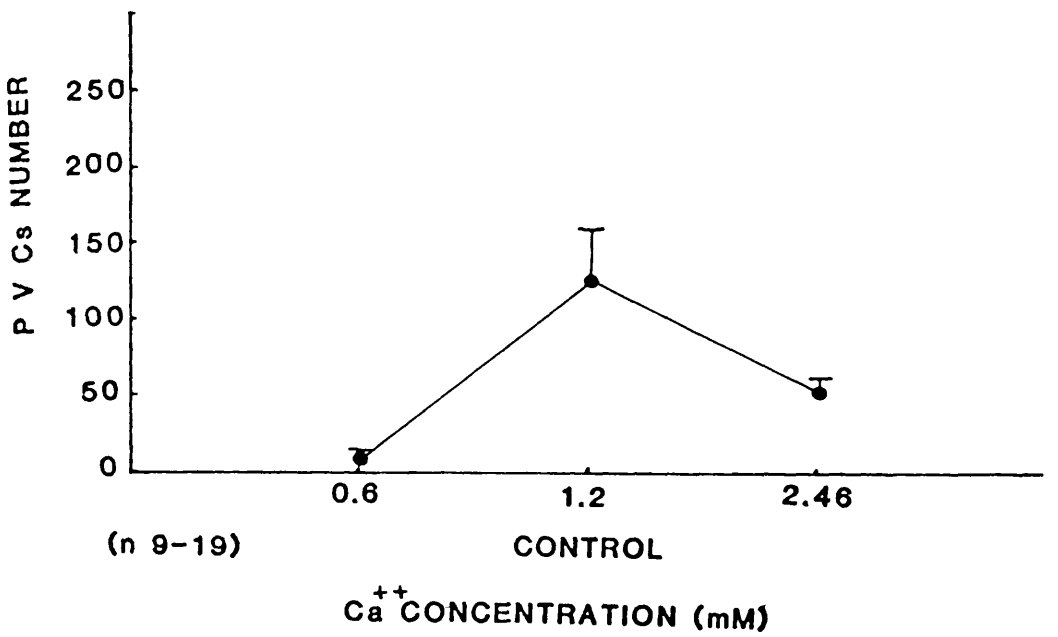


Fig. (36). Effect of calcium on the number of PVCs developed during reperfusion in the isolated rat heart. Potassium 5.9 mM; magnesium 1.2 mM.

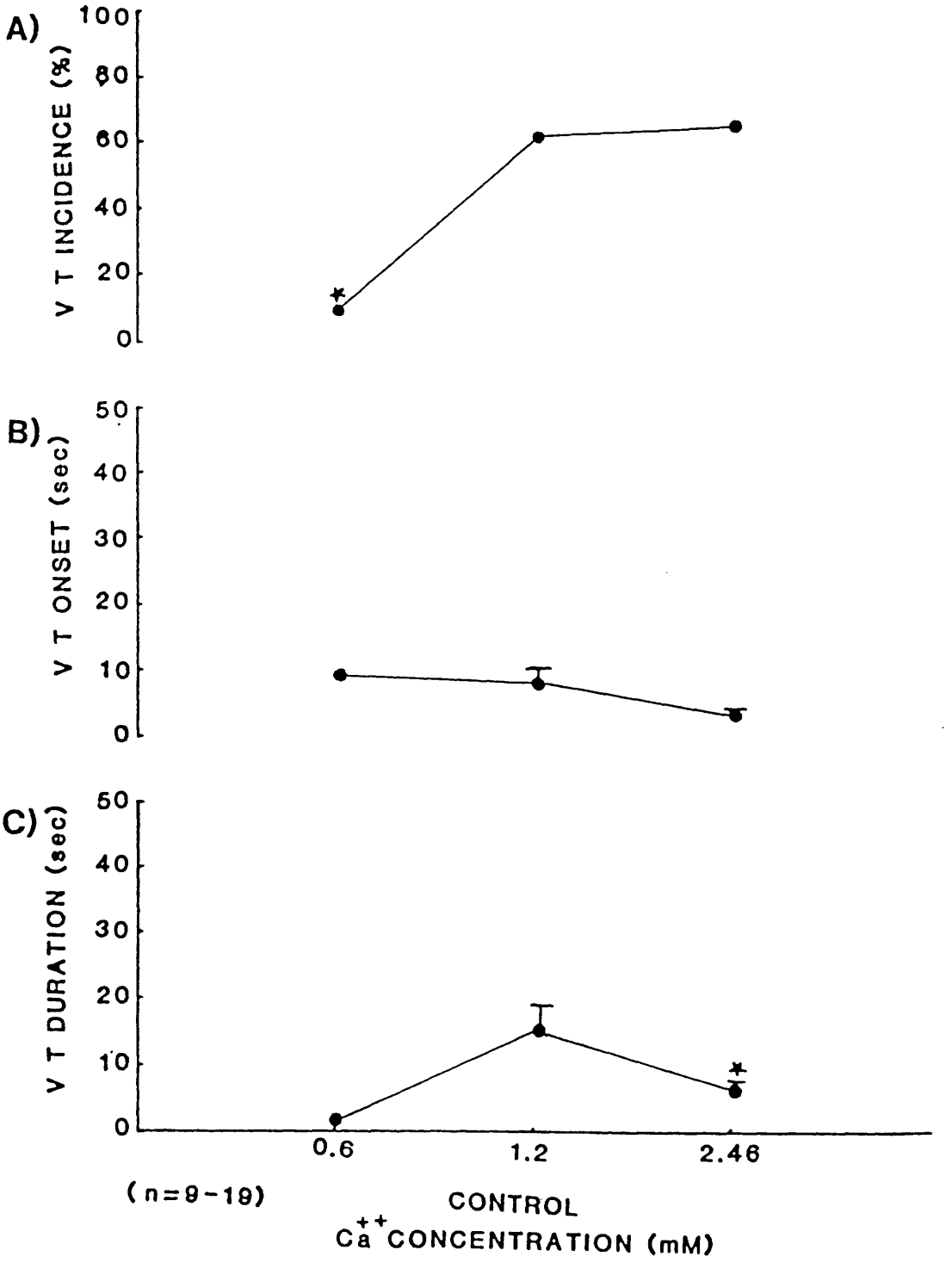


Fig. (37). Effects of calcium on a) the incidence, b) onset and c) duration of VT developed during reperfusion in the isolated rat heart. Potassium 5.9 mM; magnesium 1.2 mM .

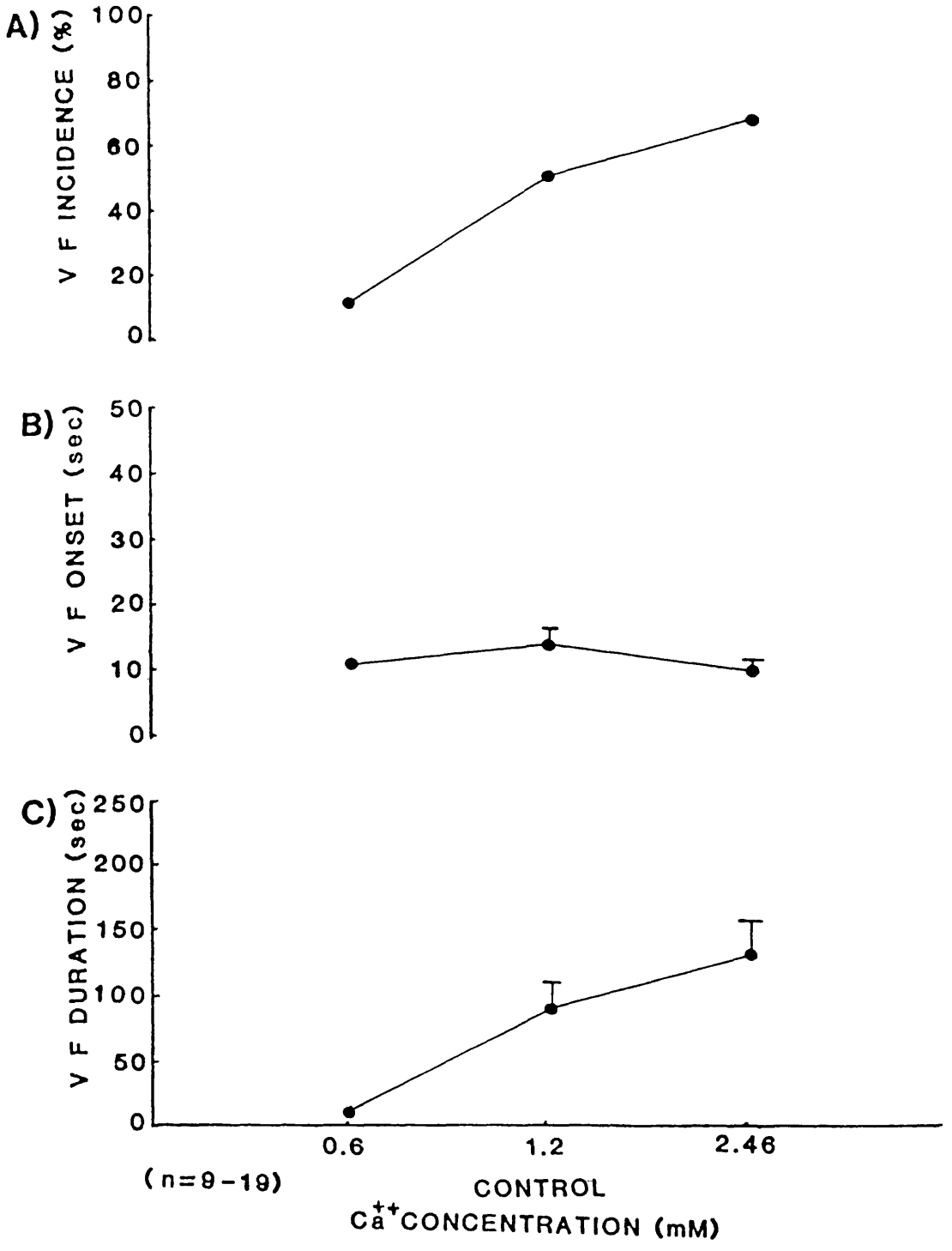


Fig. (38). Effects of calcium on a) the incidence, b)onset and c) duration of VF developed during reperfusion in the isolated rat heart. Potassium 5.9 mM; magnesium 1.2 mM.

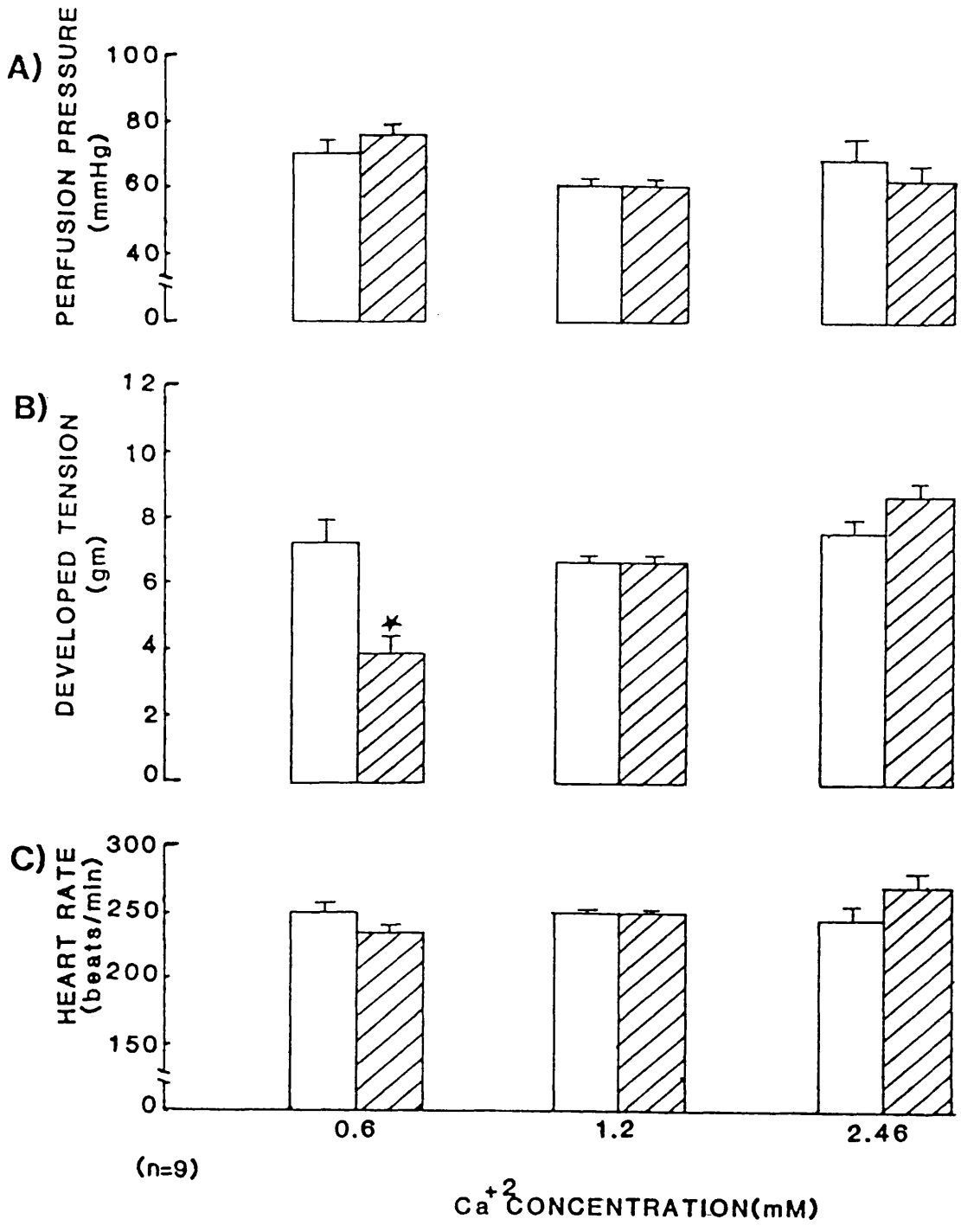


Fig. (39). Effects of calcium on a) perfusion pressure, b) developed tension and c) heart rate in the isolated rat heart. Potassium 5.9 mM; magnesium 1.2 mM.

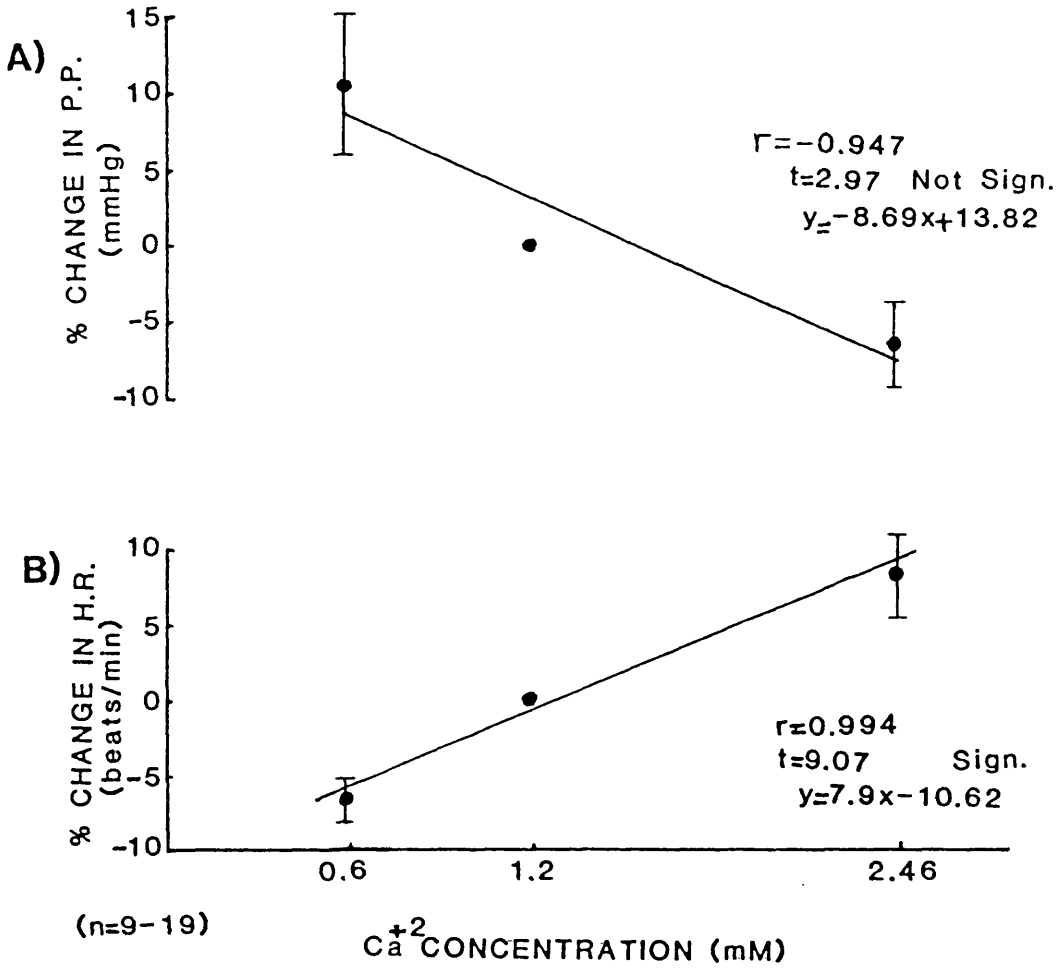


Fig. (40). Correlation between calcium concentration and a) change in perfusion pressure % and b) change in heart rate % in the isolated rat heart. Potassium 5.9 mM; magnesium 1.2 mM.

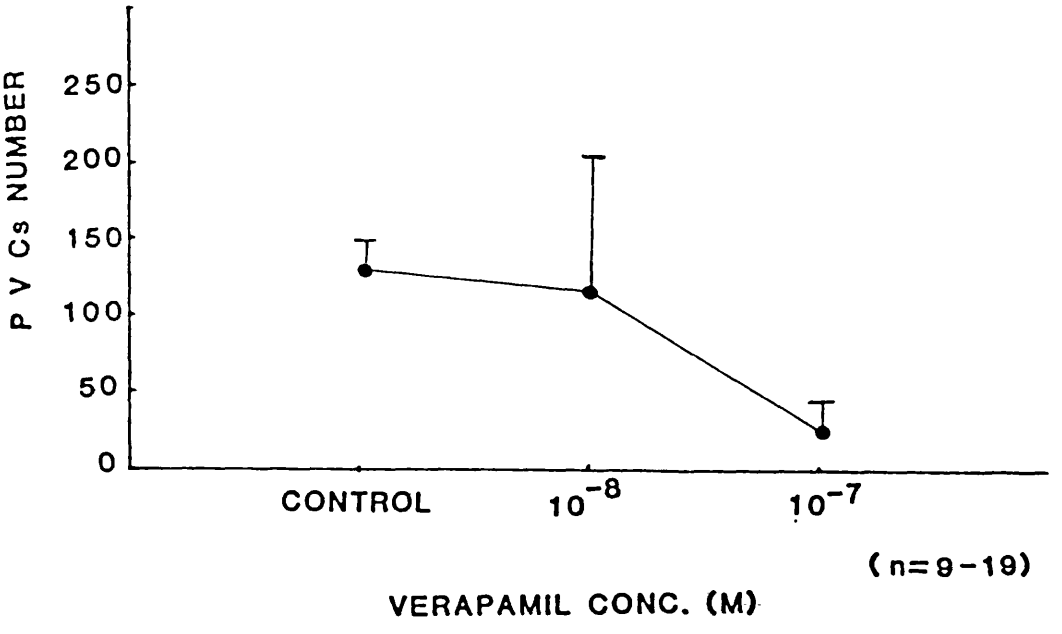


Fig. (41). Effect of verapamil (10^{-8} and 10^{-7} M) on the number of PVCs developed during reperfusion in the isolated rat heart. Potassium 5.9 mM; magnesium 1.2 mM and calcium 1.2 mM.

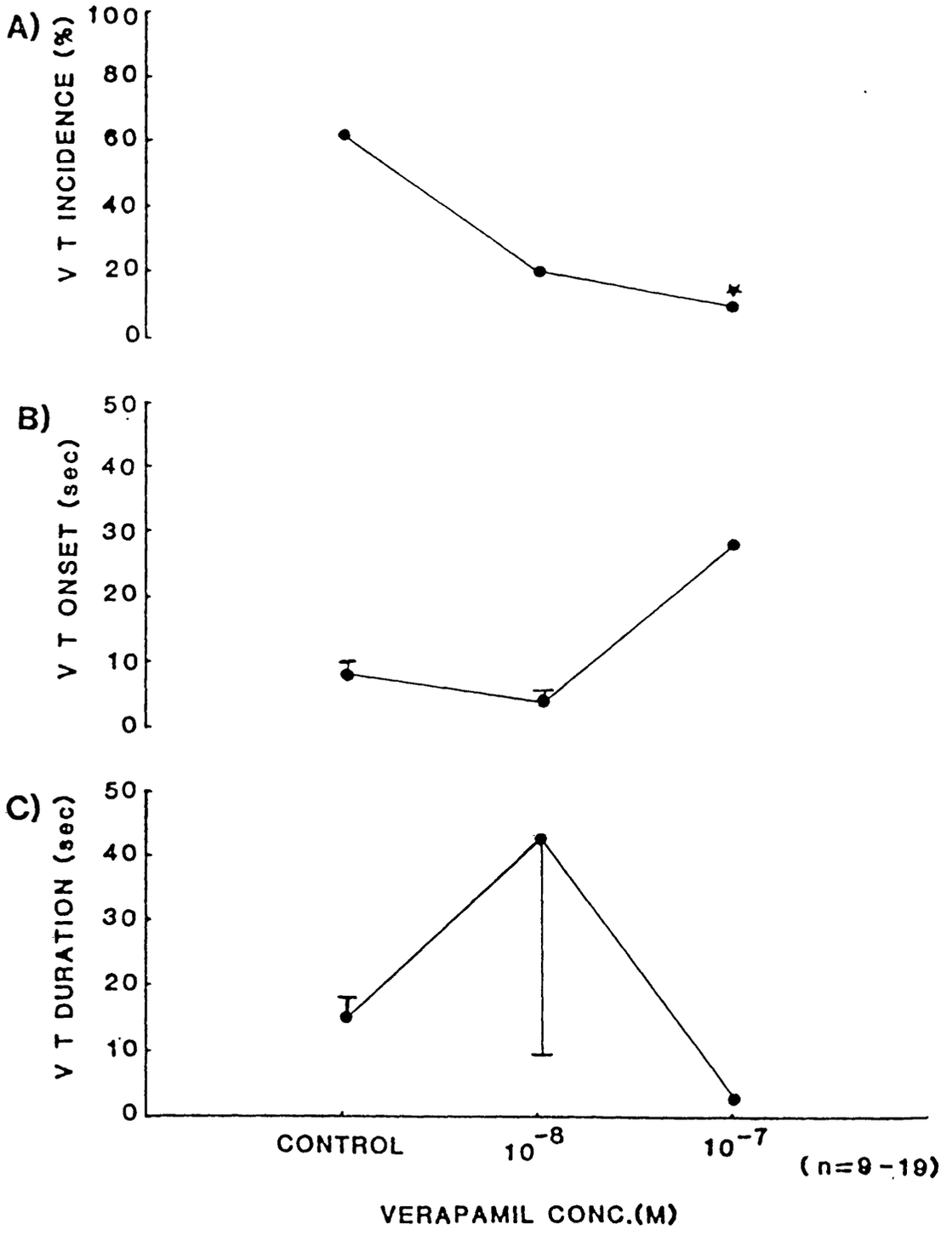


Fig. (42). Effects of verapamil (10^{-8} and 10^{-7} M) on a) the incidence, b) onset and c) duration of VT developed during reperfusion in the isolated rat heart. Potassium 5.9 mM; magnesium 1.2 mM and calcium 1.2 mM.

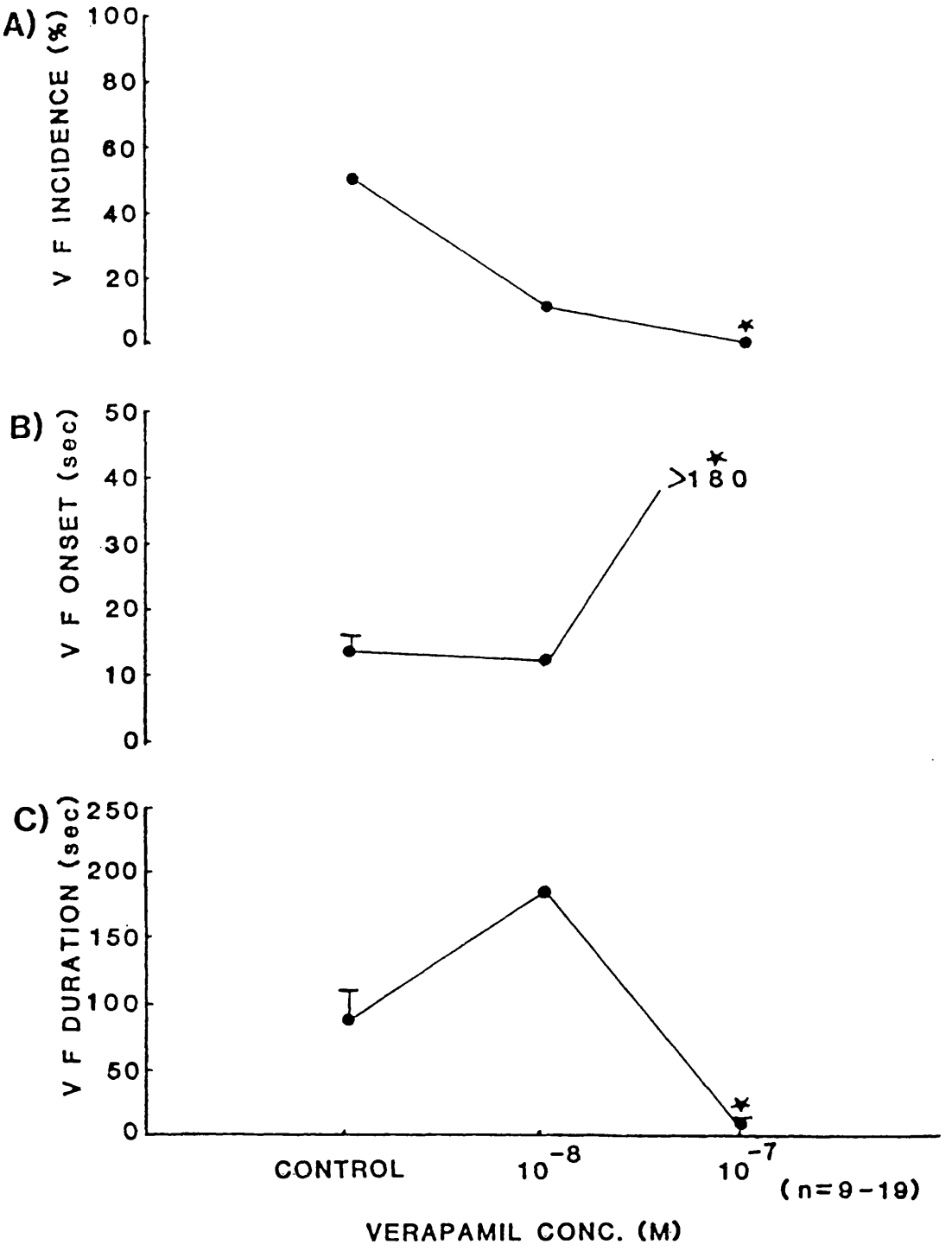


Fig. (43). Effects of verapamil (10^{-8} and 10^{-7} M) on a) the incidence, b) onset and c) duration of VF developed during reperfusion in the isolated rat heart. Potassium 5.9 mM, magnesium 1.2 mM and calcium 1.2 mM.

the duration (Fig. (43c)) of VF was increased and decreased significantly by increasing verapamil concentration to 10^{-7} M. Verapamil produced no significant change in the number of PVCs (Fig. (41)) or in the onset and duration of VT (Fig. (42 b and c)).

4.12: Haemodynamic effects of verapamil

Verapamil (10^{-8} and 10^{-7} M) produced concentration dependent reduction in perfusion pressure and developed tension (Fig. (44a and b)). Although changes in H.R. were small there was a significant negative correlation between verapamil concentration and % change in heart rate (Fig. (45b)).

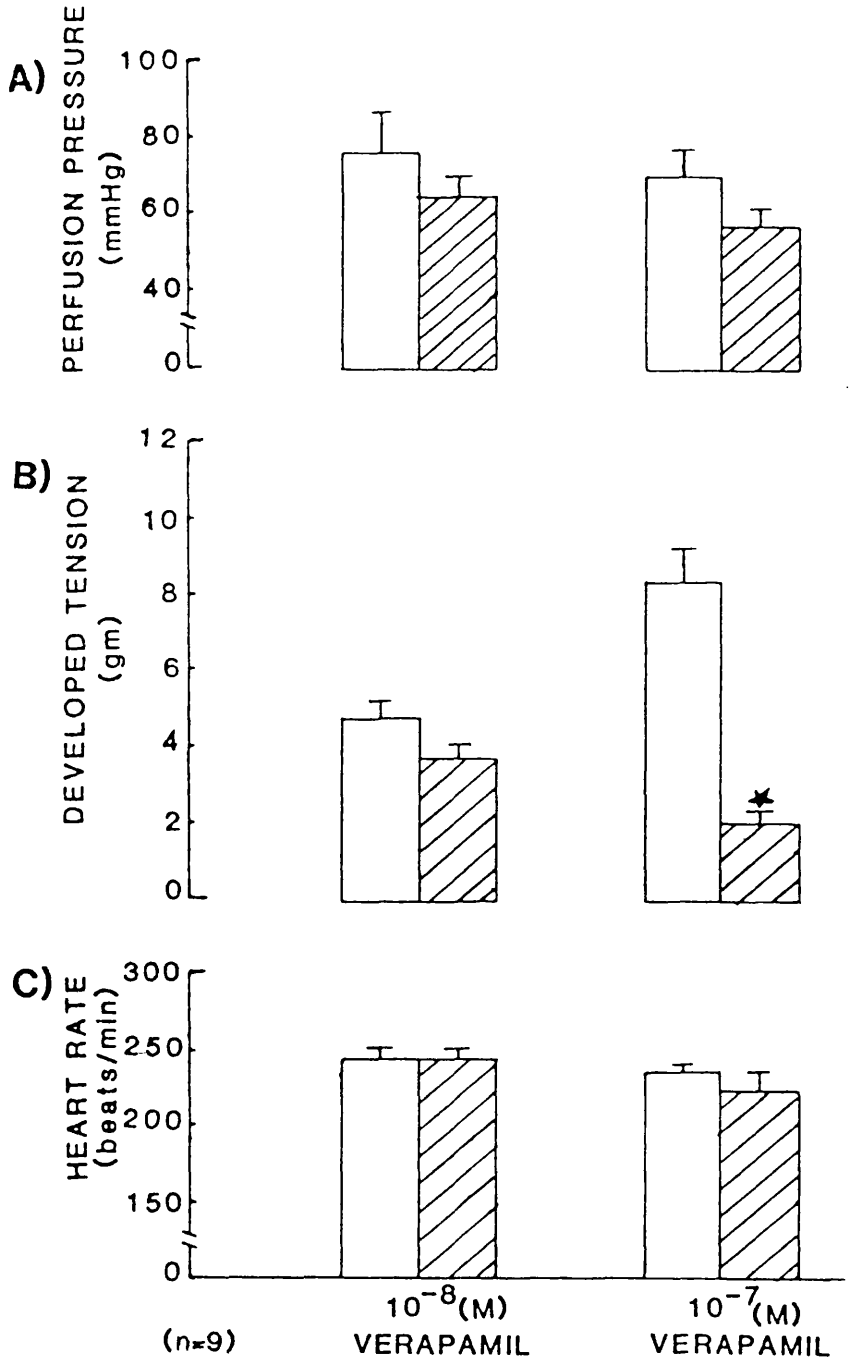


Fig. (44). Effects of verapamil (10^{-8} and 10^{-7} M) on a) perfusion pressure, b) developed tension and c) heart rate in the isolated rat heart. Potassium 5.9 mM; magnesium 1.2 mM and calcium 1.2 mM.

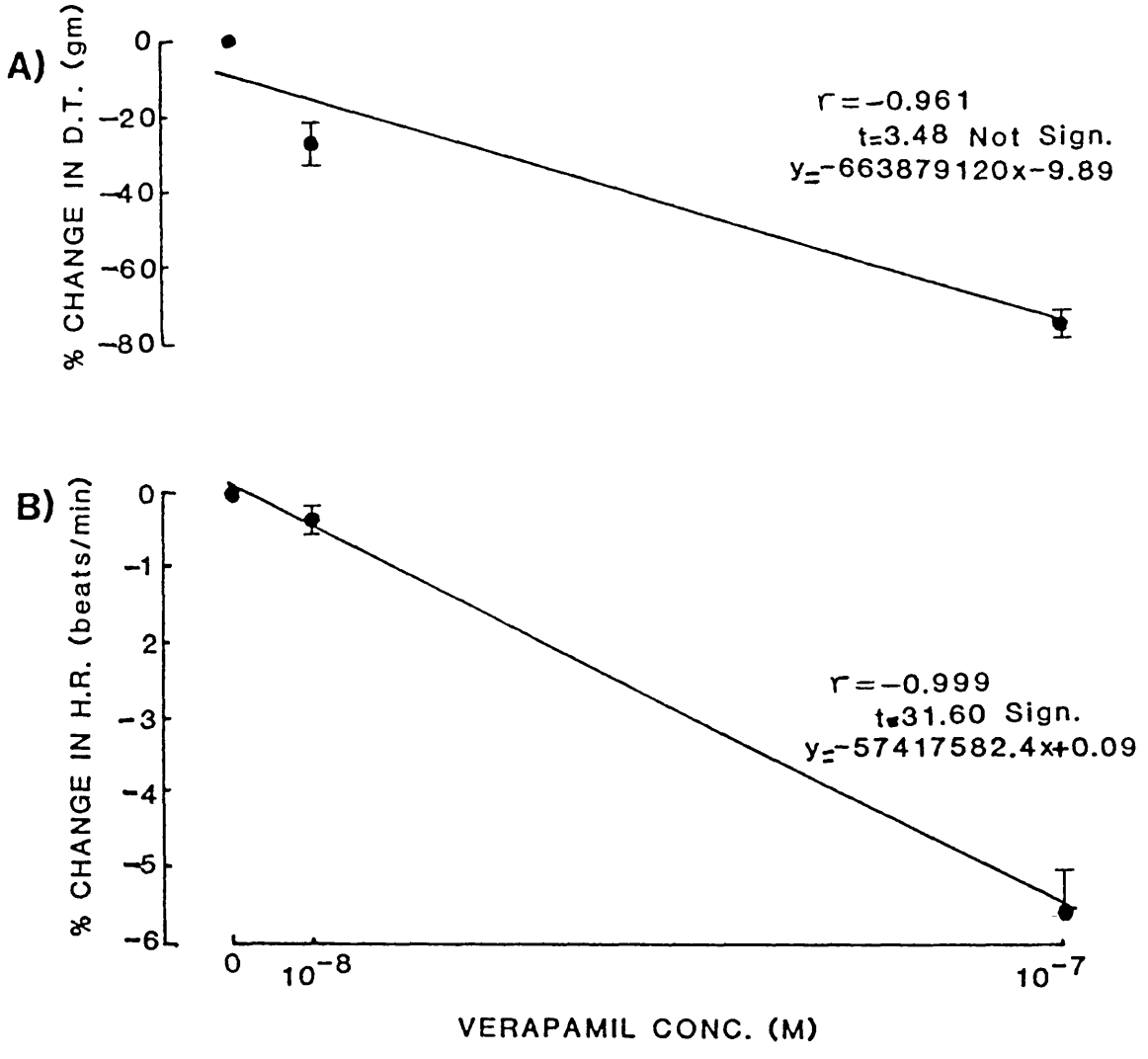


Fig. (45). Correlation between verapamil concentration (M) and a) change in developed tension % and b) change in heart rate %. Potassium 5.9 mM; magnesium 1.2 mM and calcium 1.2 mM.

Section B: Discussion

The results of this study demonstrate that the severity of reperfusion induced arrhythmias in the isolated rat heart are dependent on the ionic composition of the perfusate. Potassium (2.5 to 10 mM) and/or magnesium (0 to 4.8 mM) attenuated these arrhythmias, while calcium (0.6 to 2.4 mM) exacerbated them.

The present study confirms and extends what has previously been shown by Lubbe and his colleagues (1978) that potassium (3 to 12 mM) protected the isolated rat heart against reperfusion induced arrhythmias. In the present study a constant flow system was used while Lubbe *et al.* (1978) used constant perfusion pressure system. The major effect of potassium on the haemodynamic parameters recorded was to cause a concentration dependent fall in perfusion pressure (Fig. (27a)) which is a measure of vasodilation. Using the constant perfusion pressure system described by Lubbe *et al.* (1978), this vasodilator action of potassium would increase coronary flow. The fact that potassium reduces reperfusion induced arrhythmias in both constant pressure (Lubbe *et al.*, 1978) and constant flow systems shows that potassium is exerting an anti-arrhythmic action independent of coronary flow changes. This is in agreement with the findings of Winslow *et al.* (1983) in the isolated rat heart model perfused at a constant pressure that protection against reperfusion induced arrhythmias does not relate to coronary vasodilation. However, when the results of Lubbe *et al.* (1978) are compared with the results in this study, it is clear that potassium does exert a greater beneficial

action when a constant flow system is used which could be due to an effect on the rate of reperfusion. Dilating coronary vessels in a constant flow system reduces perfusion pressure and this will reduce the speed of reperfusion. Slow reperfusion in the isolated rat heart (Figs. (33) to(35)) and intermittent reperfusion in the dog (Sewell *et al.*, 1955) has been shown to reduce the severity of reperfusion induced arrhythmias. Therefore, the beneficial effect of potassium is probably the result of a direct antiarrhythmic action together with an effect on the rate of reperfusion. Because potassium had a positive inotropic and no chronotropic effect (Fig. 27) the beneficial effect of potassium could not be attributed to a reduction of oxygen requirements which would reduce the severity of ischaemia. In support of this, Winslow *et al.* (1983) reported that there was no correlation between negative inotropic effect produced by nifedipine or nitrendipine and protection against reperfusion induced arrhythmias in the isolated rat heart perfused at a constant pressure.

Potassium has very complex effects on the electrical activity in the nonischaemic myocardium depending on the concentration used. In heterogenous ischaemic and reperfused cardiac tissues the electrophysiological effects of potassium have not been adequately studied, therefore it is not possible to identify the mechanism of the antiarrhythmic action of potassium in this situation. Postligation increase in potassium (10 mm) protected against reperfusion arrhythmias. This, together with the fact that there is a very little collateral flow in the rat heart means that potassium would not be expected to reach the core of the ischaemic region. Therefore, the site of action of potassium

in this situation is likely to be in the nonischaemic tissue or at the border of the ischaemic/nonischaemic region, possibly by an action on the re-entry pathways which are thought to be the cause of reperfusion induced arrhythmias (Janse, 1982) by the replacement of potassium lost from the borderline of the ischaemic zone where perfusion is only reduced but not prevented.

In a recent study by Ward and Cameron (1985) it has been shown that during chronic dietary K^+ depletion there was no cellular K^+ loss from cardiac muscle and it has been suggested to be due to an adaptive change to the cardiac cell Na^+ pump during chronic K^+ depletion. This observation could not argue against the previous explanation as this adaptive change in Na^+ pump activity has not been shown to occur within minutes after K^+ depletion.

Another possible explanation of the beneficial effect of potassium is that a high potassium concentration in the perfusate reduces the steepness of the gradient of potassium from the normal to ischaemic zone (Lubbe *et al.*, 1978; Nayler,1981a). It is also possible that the protective effect of potassium against reperfusion induced arrhythmias is due toits effect on latent pacemakers as extracellular potassium concentrations above 6 - 8 mM inhibit the automaticity of the latent pacemakers (Andersson, 1980).

The increase in perfusion pressure produced on lowering perfusate potassium concentration seen in the present study may be

attributed to the fact that hypokalaemia inhibits Na^+ - K^+ ATPase enzyme leading to increase in the intracellular sodium which gives rise to the increase in intracellular calcium in coronary vessels. The decrease in perfusate potassium concentration also produces more negative resting membrane potential which shortens the action potential plateau (Gettes, 1981) leading to decrease in developed tension as shown in the present results (Fig. (27)).

Magnesium, like potassium exerted a potent antiarrhythmic action. In addition, magnesium caused a dose related bradycardia, vasodilation and negative inotropic effect. These haemodynamic actions probably contribute indirectly to its antiarrhythmic action. Pacing hearts at 300 b.p.m. to prevent the magnesium induced bradycardia attenuated but did not prevent the antiarrhythmic action of magnesium. This reduced response in the paced hearts indicates that bradycardia itself can reduce the severity of reperfusion induced arrhythmias. However, it is possible that electrical pacing released some arrhythmogenic agent which could directly counteract the protective action of magnesium. Perfusing hearts at a constant head of pressure combined with electrical pacing also attenuated the protective action of magnesium. This method of perfusion would allow coronary flow to change as described earlier and this could result in an anti-ischaemic action which could indirectly reduce reperfusion arrhythmias. However, post-ligation administration of magnesium (4.8 mM) was also antiarrhythmic. This late increase in magnesium concentration would prevent any anti-ischaemic action of magnesium because of the low collateral flow in the rat heart. However, the negative

inotropic effect induced by magnesium could still reduce oxygen requirements leading to reduction in the severity of ischaemia. Therefore, the present study shows that while the bradycardia and vasodilation and negative inotropic effect induced by magnesium contribute indirectly to its antiarrhythmic action, there is also some direct antiarrhythmic effect. Magnesium has been reported to be antiarrhythmic in man (Iseri *et al.*, 1975) and in animals (Seelig and Heggtveit, 1974) and in the wider field of cardiac disorders magnesium deficiency may be an important risk factor (Altura and Altura, 1982). Magnesium has a number of effects on the heart which could lead to antiarrhythmic activity (Shine and Douglas, 1974; Shine, 1979; Dyckner and Wester, 1980; Whang *et al.*, 1980; Nayler, 1981a). Magnesium can reduce potassium efflux from the rat heart (Shine, 1979; Whang *et al.*, 1980; Nayler, 1981a) and when given before ligation this could reduce any detrimental effect that the washout of potassium might have during reperfusion. However, it is not known if this effect of magnesium is seen in ischaemic tissues. Also, the fact that post-ligation increase of magnesium was antiarrhythmic suggests that some other action of magnesium is more important. Magnesium causes a loss of intracellular calcium, and it can reduce the movement of extracellular calcium into the cell by competing with calcium at the sarcolemma (Shine and Douglas, 1974; Dyckner and Wester, 1980). This effect is rapid and may explain the haemodynamic effects of magnesium shown in the present study (Fig. (32)). This effect of magnesium on calcium movement may be important particularly as calcium may enter the cell during reperfusion (Shine *et al.*, 1978) and calcium overload can initiate arrhythmias (Woodward, 1981; De Mello, 1982). Although calcium accumulation does occur following reperfusion and reoxygenation,

this has only been reported with periods of ischaemia longer than the 10 min used in the present study, therefore this explanation of the action of magnesium is only speculative.

In support of the above suggestion, it was demonstrated that increasing calcium concentrations (0.6 to 2.4 mM) did exacerbate reperfusion induced arrhythmias in this model, also, VF was associated with a sudden increase in resting tension which is indicative of a rise in intracellular free calcium. In the present study, the major haemodynamic effects of increased calcium were to increase developed tension and heart rate. These haemodynamic effects of calcium could indirectly increase the severity of the arrhythmias by exacerbating the ischaemic damage. Reduction of the contractility and heart rate by lowering of calcium concentration may reduce tissue demand for oxygen and high energy phosphates thereby preventing tissue damage. Decreasing calcium influx might also spare high energy phosphates required for calcium sequestration and attenuate calcium induced-injury of cellular organelles due to activation of phospholipases or calcium overloading of the mitochondria (Shine and Douglas, 1983). Also low calcium perfusate may reduce gap junctional resistance leading to improved conduction and protection against arrhythmias (De Mello, 1982). In agreement with the data in the present study, Shine *et al.* (1978) have shown that reperfusion of rabbit interventricular septa after 20 min and 60 min of ischaemia with a solution containing a reduced calcium concentration (0.75 mM) protected against ischaemic injury.

Studies with calcium slow channel blocking drugs have provided

conflicting results as to their efficacy in preventing reperfusion induced arrhythmias. Nifedipine has been shown to be effective in preventing reperfusion induced arrhythmias in one study (Parratt, 1982) but not in others (Winslow *et al.*, 1983; Kane *et al.*, 1984). Similarly, verapamil has been shown to be effective in preventing reperfusion induced arrhythmias in the present results and other studies (Parratt, 1982; Winslow *et al.*, 1983; Bergey *et al.*, 1984) but not in others (Kane *et al.*, 1984). These conflicting pharmacological reports suggest that if calcium accumulation is involved in the development of reperfusion induced arrhythmias then calcium slow channel is not the major route by which calcium enters into the cell.

The changes in magnesium and calcium concentration used in the present study would not be seen in man unless an extreme pathological condition existed. On the contrary, it is clear that potassium over the physiological range 2.5 mM to 5.9 mM did have a marked effect on reperfusion induced arrhythmias in the isolated rat heart. If reperfusion arrhythmias are a cause of sudden death in man then hypokalaemia could be a serious risk factor. Recent continuous measurements of intravascular potassium show that potassium levels can change very rapidly during and after exercise or catecholamine administration (Band *et al.*, 1982; Linton *et al.*, 1982). If a transient hypokalaemia occurred in the presence of mild hypomagnesaemia or hypercalcaemia then these changes in magnesium and/or calcium could be important factors in determining the severity of any reperfusion arrhythmias that might occur. The present results suggest that perfusion pressure, developed tension, heart rate and the rate of reperfusion can affect the

severity of reperfusion induced arrhythmias. As hypokalaemia (Dietz, 1983) and hypomagnesaemia (Altura and Altura, 1982) are risk factors for the development of hypertension, and as hypertension is a risk factor for cardiac disease, this suggests that maintaining plasma potassium and magnesium levels at the higher end of the physiological range may be beneficial indirectly in preventing these arrhythmias via a hypotensive action as well as by any direct action on the heart.

CHAPTER 5

"Effect of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart

Section A: Results

The effects of changes in K^+, Mg^{2+} and Ca^{2+} distribution between the intra and extracellular compartments mentioned in the previous chapter may be induced by the generation of oxygen free radical on reperfusion of the ischaemic myocardium. Free radicals cause peroxidation of lipids in the cell membrane (see Chapter 1.11) which will alter the ionic permeability of the sarcolemma. They also affect intracellular calcium sequestration (Chapter 1.11) and these changes might be expected to lead to the development of arrhythmias. Therefore,it was of interest to study the effect of some free radical scavengers on reperfusion induced arrhythmias. In addition, experiments have been carried out to try to detect superoxide in the perfusate of these rat hearts. A potassium concentration of 3.2 mM was used in these experiments to increase the incidence of reperfusion induced arrhythmias.

A) Effect of some free radical scavengers on reperfusion arrhythmias and their haemodynamic effects

5.1: Superoxide dismutase

Superoxide dismutase (5, 10 and 20 units. ml^{-1}) reduced the incidence of reperfusion induced VF in a concentration dependent manner (Table 8) while it had no significant effect on the number of PVCs or the incidence of VT. At the concentrations used superoxide dismutase had no effect on perfusion pressure, developed tension or heart rate. There was a significant negative correlation between

Table 8. Effects of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart.

	n	PVCs/3 min	VT			VF		
			incidence (%)	onset (sec)	duration (sec)	incidence (%)	onset (sec)	duration (sec)
Control	21	142±32	86	12±4	13±3	91	21±5	99±16
SOD (a) 5 u/ml	9	136±38	78	14±3	14±3	56	20±4	67±40
(b) 10 u/ml	9	57±20	56	40±28	9±3	33*	17±3	161±6
(c) 20 u/ml	9	61±17	56	19±7	9±1	22*	16±1	84±81
Glutathione:								
(a) 10^{-5} M	9	257±115	78	8±3	30±14	67	22±7	106±29
(b) 10^{-4} M	9	142±38	78	21±5	14±3	22*	22±6	90±62
(c) 10^{-3} M	9	121±38	56	67±18	13±5	0*	-*	-*
Catalase:								
(a) 100 u/ml	9	102±29	67	32±10	12±3	56	49±22	36±30
(b) 300 u/ml	9	82±19	78	12±3	9±2	56	20±3	90±29
Mannitol:								
(2×10^{-2} M)	9	64±13	89	14±3	7±2	56	20±2	100±38
Ascorbic acid								
(a) 10^{-4}M	9	113±29	78	17±6	19±5	67	30±7	105±32
(b) 5×10^{-4} M	9	127±40	67	12±2	20±6	22*	22±3	10±3*
Histidine:								
(a) 10^{-3}M	9	114±21	89	12±3	8±1	67	16±3	59±34
(b) 5×10^{-3}M	9	113±47	44*	12±4	15±6	0*	-*	-*

Drugs were administered 5 min before coronary ligation.

* $P < 0.05$ compared with controls.

Control: 3.2 mM K^+, 1.2 mM Mg^{2+} and 1.2 mM Ca^{2+}.

concentration of superoxide dismutase and the incidence of VF (Fig. (46)).

5.2: Glutathione

Glutathione significantly reduced the development of VF at the concentration of 10^{-4} M and completely prevented it at the highest concentration 10^{-3} M. Glutathione had no significant effect on the incidence, onset and duration of VT, the number of PVCs (Table 8) or the heart rate. Although glutathione was used in the present study in relatively high concentrations (10^{-5} - 10^{-3} M), these concentrations used are similar to the normal tissue levels of glutathione.

5.3: Ascorbic acid

Ascorbic acid can act as a reducing agent and can prevent lipid peroxidation induced by the hydroxyl, superoxide and singlet oxygen radicals. Ascorbic acid (5×10^{-4} M) reduced the incidence and duration of VF while it had no significant effect on the number of PVCs or the incidence, onset and duration of VT (Table 8). The two concentrations of ascorbic acid used significantly increased the perfusion pressure while having no effect on developed tension or heart rate (Fig. (47)).

5.4: Histidine

Histidine at a concentration of 5×10^{-3} M reduced the incidence of VT and completely prevented the development of VF. It had no significant effect on the number of PVCs or the onset and duration of VT. Histidine at the lower concentration 10^{-3} M

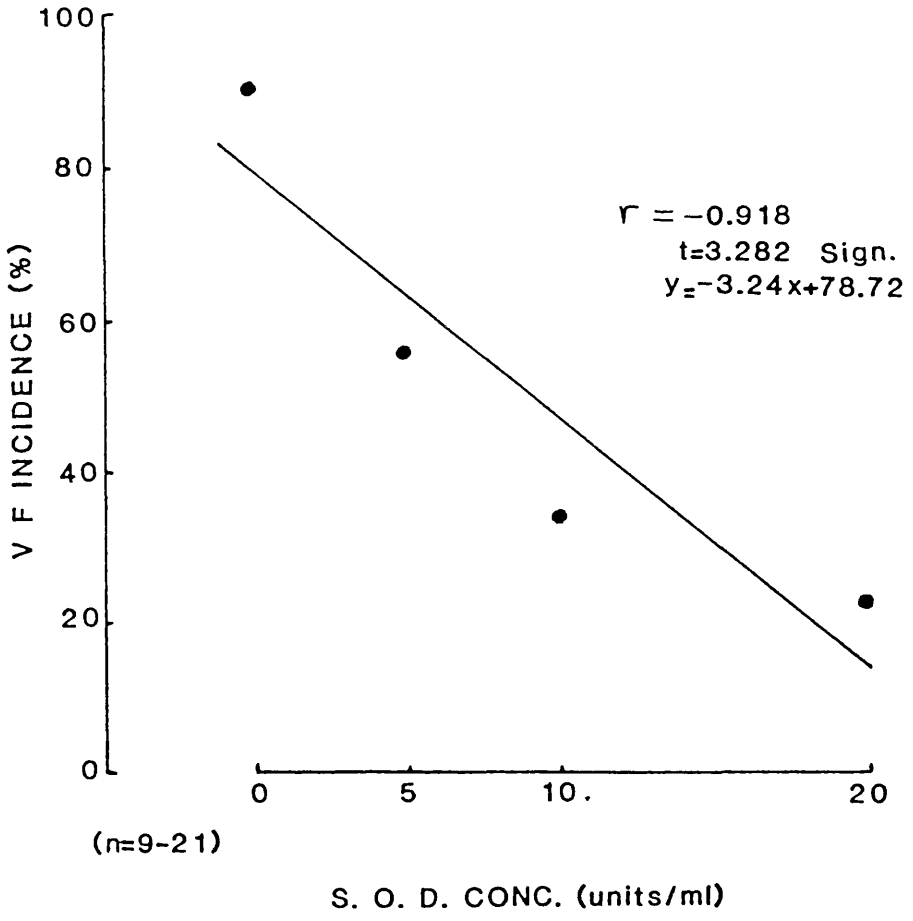


Fig. (46). Correlation between superoxide dismutase concentration (units. ml^{-1}) and the incidence % of VF developed on reperfusion. Potassium 3.2 mM; magnesium 1.2 mM and calcium 1.2 mM.

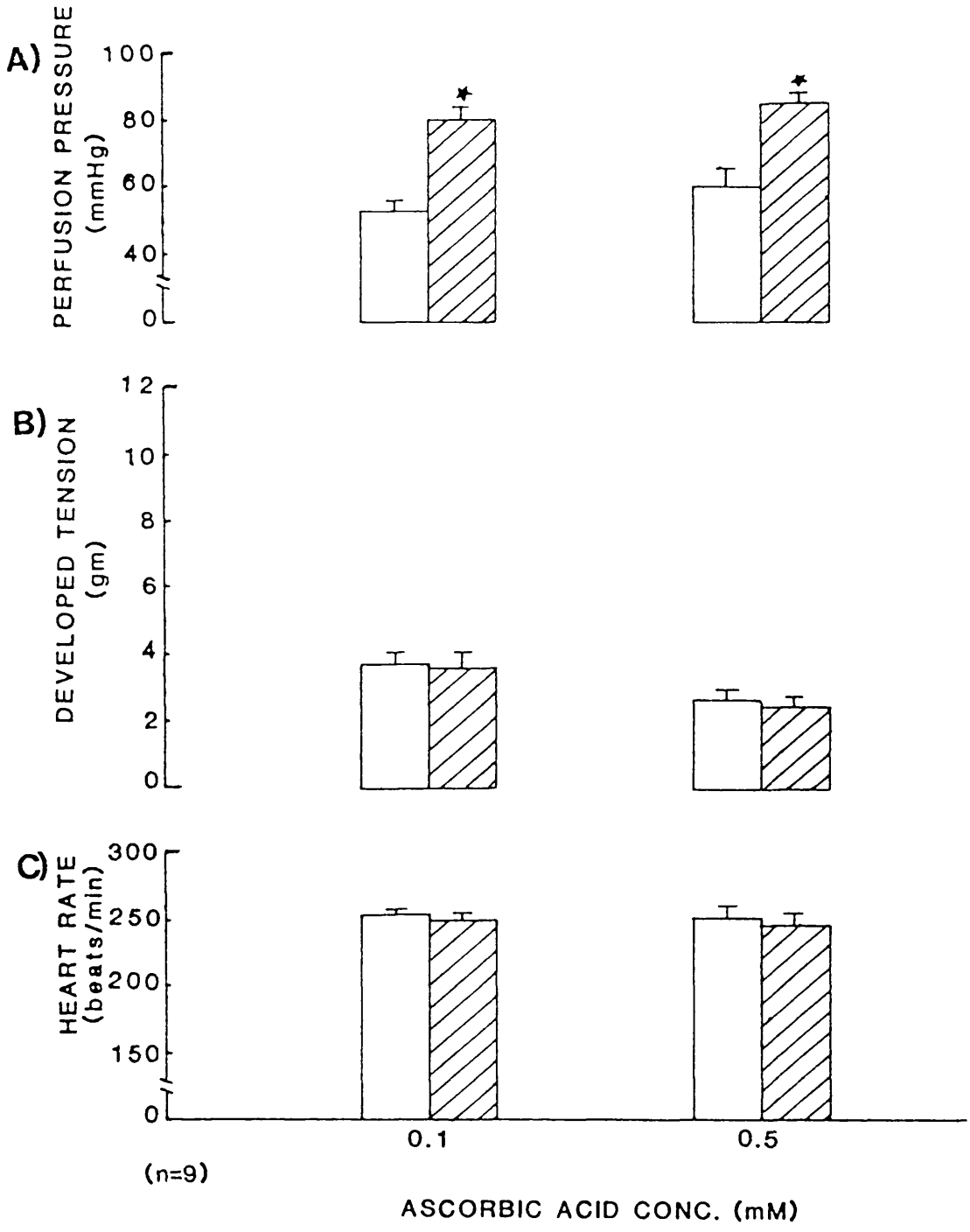


Fig. (47). Effects of ascorbic acid (10^{-4} and 5 x 10^{-4} M) on (a) perfusion pressure, (b) developed tension and (c) heart rate in the isolated rat heart. Potassium 3.2 mM; magnesium 1.2 mM and calcium 1.2 mM.

had no significant effect on the severity of arrhythmias (Table 8). Histidine produced concentration dependent vasoconstrictor and negative inotropic effects (Fig. (48)).

5.5: Catalase and mannitol

At the concentrations used catalase and mannitol had no significant effect on the development of arrhythmias when given alone (Table 8). When these two drugs were given in combination they produced a significant reduction in the incidence of VF while they had no significant effect on the number of PVCs, the incidence of VT or the onset and duration of VT and VF (Table 9). Catalase and mannitol had no significant effect on perfusion pressure, developed tension or heart rate when given either alone or in combination.

5.6: Superoxide dismutase plus catalase and/or mannitol

As catalase and mannitol had an additive antiarrhythmic action, it was of interest to see if these drugs alone, or in combination could potentiate the effects of superoxide dismutase. As it can be seen from Table (9) the combination of superoxide dismutase (10 units. ml^{-1}) and catalase (100 units.ml^{-1}) produced a greater reduction in the incidence of VF than either drug alone. A similar additive effect was seen with the combination of superoxide dismutase (10 units.ml^{-1}) and mannitol (2×10^{-2} M) (Table 9). When all three drugs were given in combination this proved to be a very potent antiarrhythmic mixture. Ventricular fibrillation was completely prevented while the number of PVCs and the incidence of VT were also significantly reduced (Table 9). The combinations

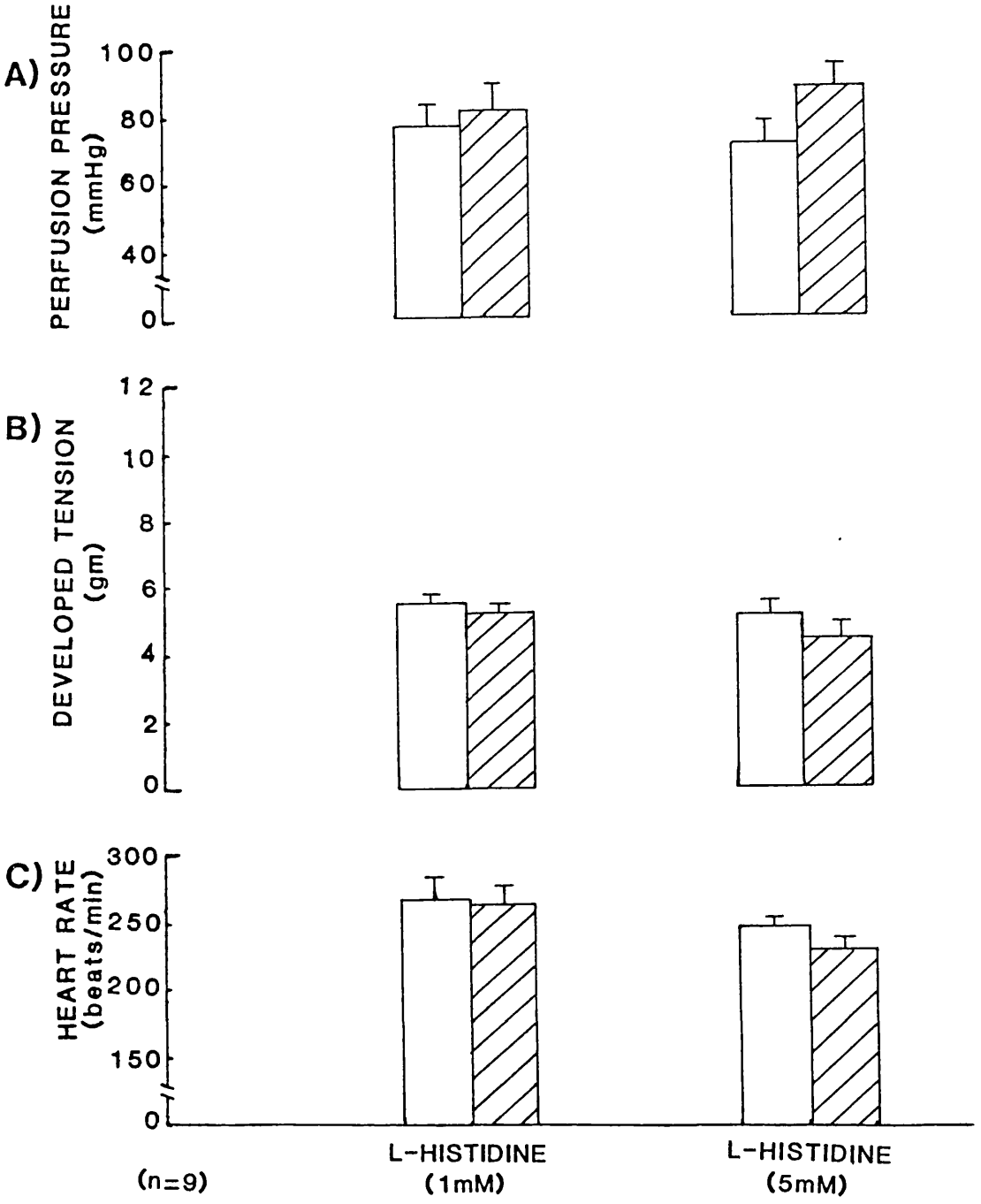


Fig. (48). Effects of histidine (10^{-3} and 5 x 10^{-3} M) on (a) perfusion pressure, (b) developed tension and (c) heart rate in the isolated rat heart. Potassium 3.2 mM; magnesium 1.2 mM and calcium 1.2 mM.

Table 9. Effects of superoxide dismutase, catalase and mannitol alone and in different combinations on reperfusion induced arrhythmias in the isolated rat heart.

	n	PVCs/ 3 min	VT			VF		
			incidence (%)	onset (sec)	duration (sec)	incidence (%)	onset (sec)	duration (sec)
Control	21	142±32	86	12±4	13±3	91	21± 5	99± 16
SOD (10 u/ml)	9	57±20	56	40±28	9±3	33*	17± 3	161± 6
Catalase (100u/ml)	9	102±29	67	32±10	12±3	56	49±22	36±30
Mannitol($2x10^{-2}$M)	9	64±13	89	14±3	7±2	56	20±4	100±38
SOD (10 u/ml) + Catalase (100 u/ml)	9	58±38*	33*	11±5	13±9	22*	20±8	18±15
SOD (10 u/ml + Mannitol ($2x10^{-2}$M)	9	96±59	56	67±29	19±13	11*	19	161
Catalase (100u/ml) + Mannitol ($2x10^{-2}$M)	9	119± 41	56	15± 2	22± 7	33*	36± 11	60± 49
SOD (10 u/ml) + Catalase (100 u/ml) + Mannitol (2x10-2 M)	9	20±10*	22*	20±4	6±2	0*	-*	-*

Drugs were administered 5 min before coronary ligation. * $P < 0.05$ compared with controls.

Control: 3.2 mM K^+, 1.2 mM Mg^{2+} and 1.2 mM Ca^{2+}.

of all three drugs has no significant effect on perfusion pressure, developed tension or heart rate (Fig. (49)).

5.7: Effects of some free radical scavengers when given after coronary ligation

Results shown in Chapter 5.1-5 were obtained from experiments in which drugs were added 5 min before coronary artery ligation. If the drugs reduced the severity of the ischaemic damage this could indirectly reduce reperfusion arrhythmias. Therefore the antiarrhythmic action of the three most effective drug treatments was reexamined when given 2 min before reperfusion. From Figs. (50) - (52) it can be seen that glutathione (10^{-3} M), L-histidine (5×10^{-3} M) and the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units. ml^{-1}) plus mannitol (2×10^{-2} M) still reduced the incidence of reperfusion induced VF when given 2 min before reperfusion. Histidine only reduced the incidence of VT while none of them had significant effect on the number of PVCs (Fig. (50)) or the onset and duration of VT (Fig. (51)) and VF (Fig. (52)). In these experiments, five hearts treated with glutathione and eight hearts treated with superoxide dismutase/catalase/mannitol mixture, did not develop VF within 3 min of reperfusion. In order to see if VF was simply being delayed in these hearts, the reperfusion period was extended to 10 min. Of the total of 13 hearts reperfused for this length of time only one developed VF. This heart which had been treated with the superoxide dismutase/catalase/mannitol mixture had a short run (35 sec) of VF after 8 min of reperfusion. The remaining 12 hearts were very stable during the 3 to 10 min period. These results indicate that drug treatment is not simply delaying the onset of reperfusion arrhythmias.

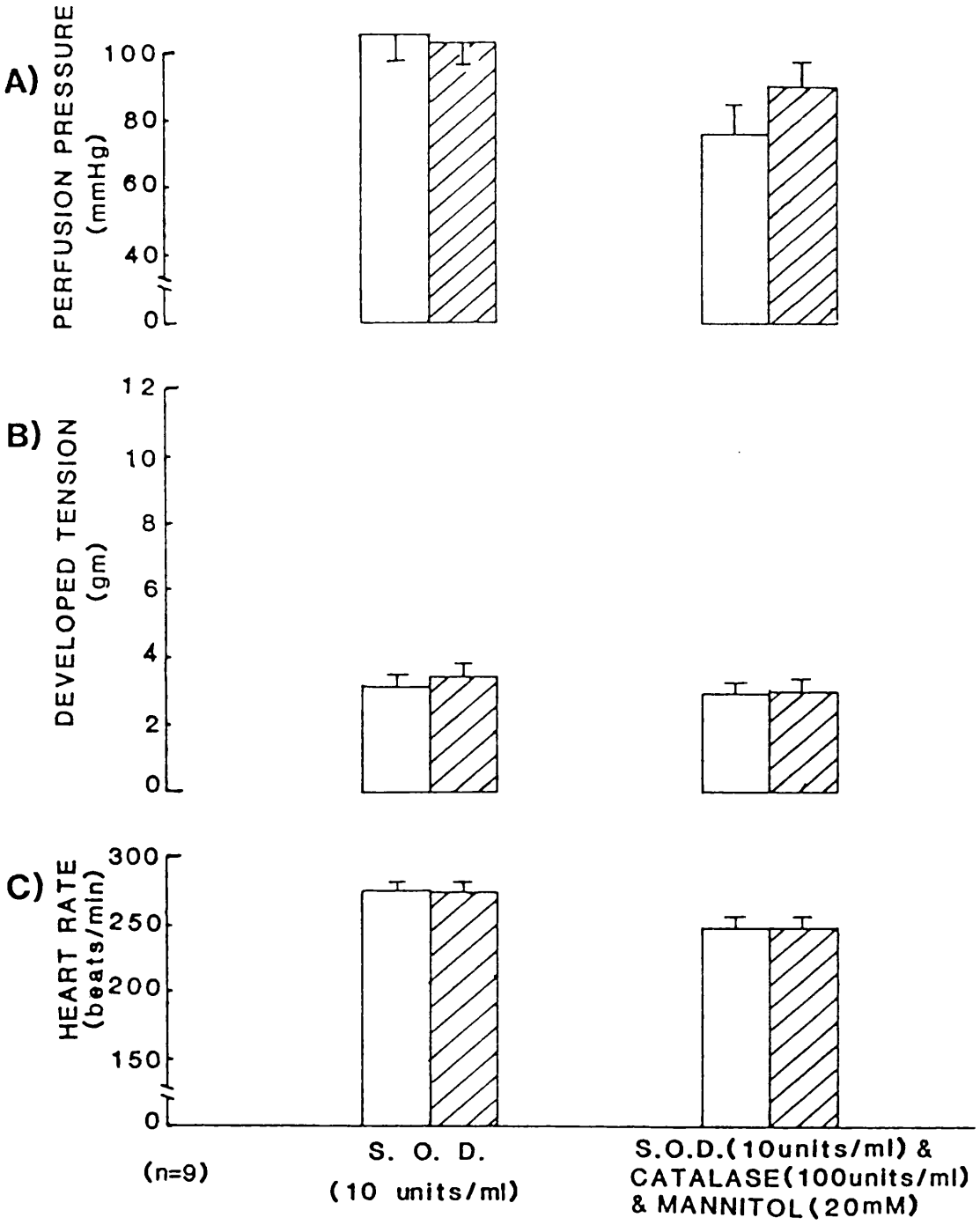


Fig. (49). Effects of superoxide dismutase (10 units.ml^{-1}) alone and in combination with catalase (100 units.ml^{-1}) plus mannitol (2 x 10^{-2} M) on a) perfusion pressure, b) developed tension and (c) heart rate in the isolated rat heart. Potassium 3.2 mM; magnesium 1.2 mM and calcium 1.2 mM.

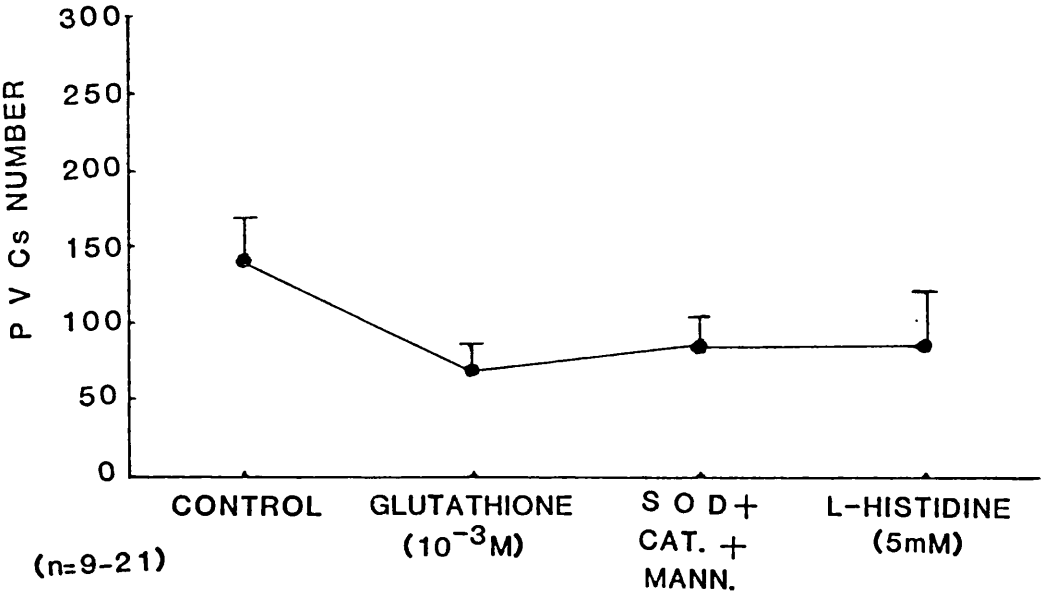


Fig. (50). Effect of glutathione (10^{-3} M), L-histidine (5×10^{-3} M) and the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) plus mannitol (2×10^{-2} M) when administered 2 min before reperfusion on the number of PVCs developed during reperfusion. Potassium 3.2 mM; magnesium 1.2 mM and calcium 1.2 mM.

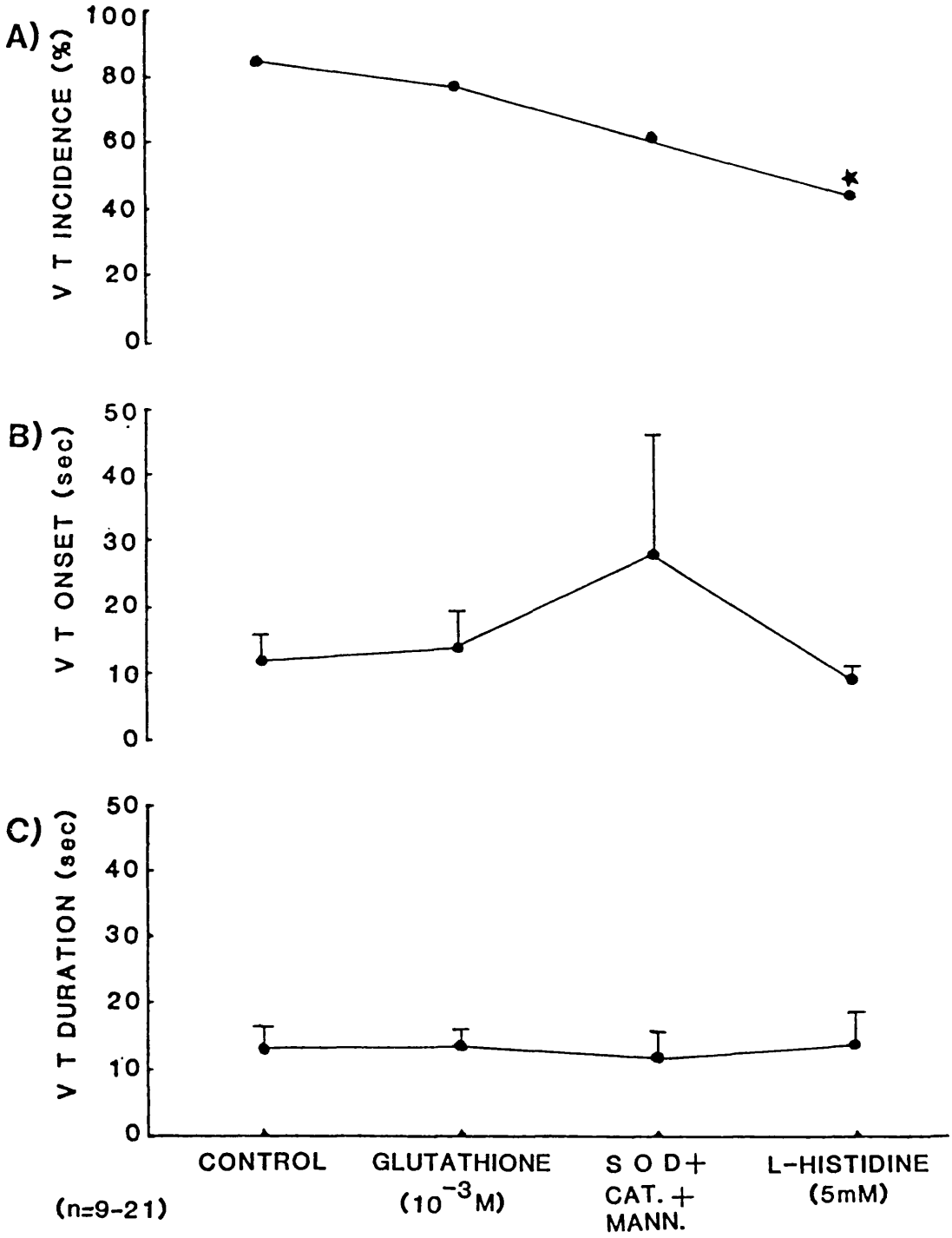


Fig. (51). Effects of glutathione (10^{-3} M), L-histidine (5 x 10^{-3} M) and the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) plus mannitol(2 x 10^{-2} M) when administered 2 min before reperfusion on (a) the incidence, (b) onset and (c) duration of VT developed during reperfusion. Potassium 3.2 mM.

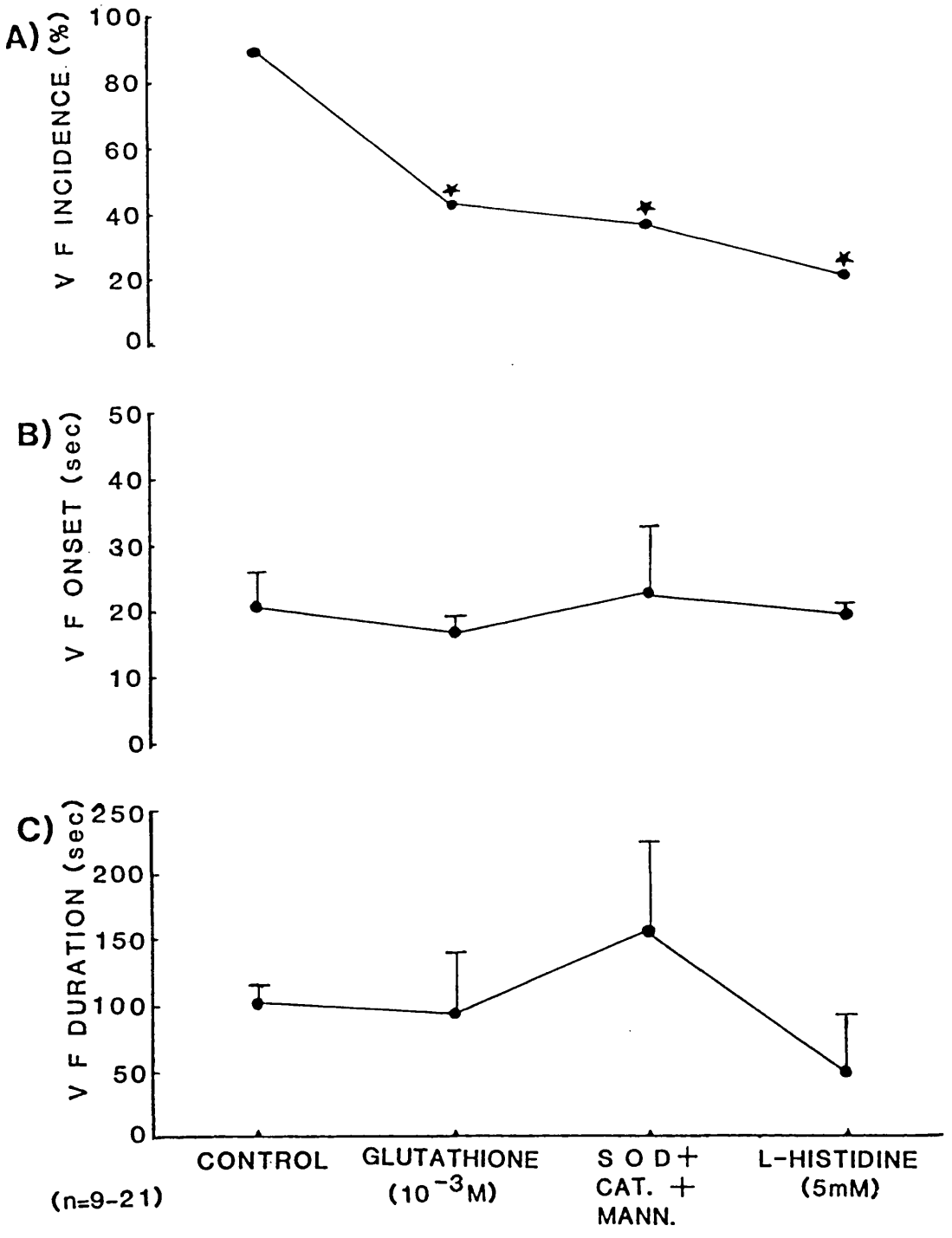


Fig. (52). Effects of glutathione (10^{-3} M), L-histidine (5×10^{-3} M) and the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) plus mannitol (2×10^{-2} M) when administered 2 min before reperfusion on (a) the incidence, (b) onset and (c) duration of VF developed during reperfusion. Potassium 3.2 mM.

B) Effects of agents which potentiate the production of free radicals

5.8: Hydrogen peroxide

Hydrogen peroxide may react with superoxide anion (Haber Weiss reaction: Chapter 1.11) in the presence of transition metals to produce the highly reactive species, the hydroxyl radical. Hydrogen peroxide was expected to increase the incidence of reperfusion induced arrhythmias, therefore, in these experiments high potassium concentration (7.5 mM) was used to reduce the incidence of reperfusion induced arrhythmias and to leave a chance for the compound under investigation to increase the incidence of arrhythmias within a reasonable range.

Hydrogen peroxide had no significant effect on the number of PVCs or the incidence, onset and duration of VT and VF (Table 10). It had a concentration dependent vasoconstrictor and positive inotropic effect over the concentration range studied (Fig. (53)). There was a significant positive correlation between hydrogen peroxide concentration and change in perfusion pressure % (Fig. (54b)).

5.9: Iron

Iron as a transition metal was expected to increase the incidence of reperfusion induced arrhythmias by increasing the production of the hydroxyl radicals via the Haber-Weiss reaction, therefore it was important to investigate the effect of iron on the incidence of reperfusion arrhythmias. In these experiments potassium concentration of 5.9 mM was used to avoid the possibility

Table 10. Effects of (A) hydrogen peroxide and (B) desferrioxamine and (C) ferric ion and ferrous ion on reperfusion induced arrhythmias in the isolated rat heart.

	n	PVCs/ 3 min	VT incidence (%)	VT onset (sec)	VT duration (sec)	VF incidence (%)	VF onset (sec)	VF duration (sec)
A) Control	9	59±42	22	9±5	22±13	22	25±12	12±5
Hydrogen peroxide								
(a) 3 x 10^{-5}M	5	43±37	20	12	24	20	28	8.5
(b) 12 x 10^{-5} M	6	75±41	50	6±1	13±6	33	24±11	65±63
B) Control	21	142±32	86	12±4	13±3	91	21±5	99±16
Desferrioxamine								
(2.5 x 10^{-4})	9	76±24	78	9±2	8±3	78	11±3	167±4
C) Control	19	127±36	63	8±2	15±3	53	14±3	88±23
Fe^{3+} (5 x 10^{-5}M)	9	133±33	67	8±1	16±3	56	20±2	11±6
Fe^{2+} (5 x 10^{-5}M)	14	262±56	100*	13±4	30±7	86*	29±6	75±20
Fe^{2+} (5 x 10^{-5}M) + Desferrioxamine (2.5 x 10^{-4}M)	9	90±31	56	5±1	11±2	56	13±3	65±37

Drugs were administered 5 min before coronary ligation. * $P < 0.05$ compared with control.

In (A) control: 7.5 mM K^+, 1.2 mM Mg^{2+} and 1.2 mM Ca^{2+}; (B) control: 3.2 mM K^+, 1.2 mM Mg^{2+} and 1.2 mM Ca^{2+}

(C) Control: 5.9 mM K^+, 1.2 mM Mg^{2+} and 1.2 mM Ca^{2+}

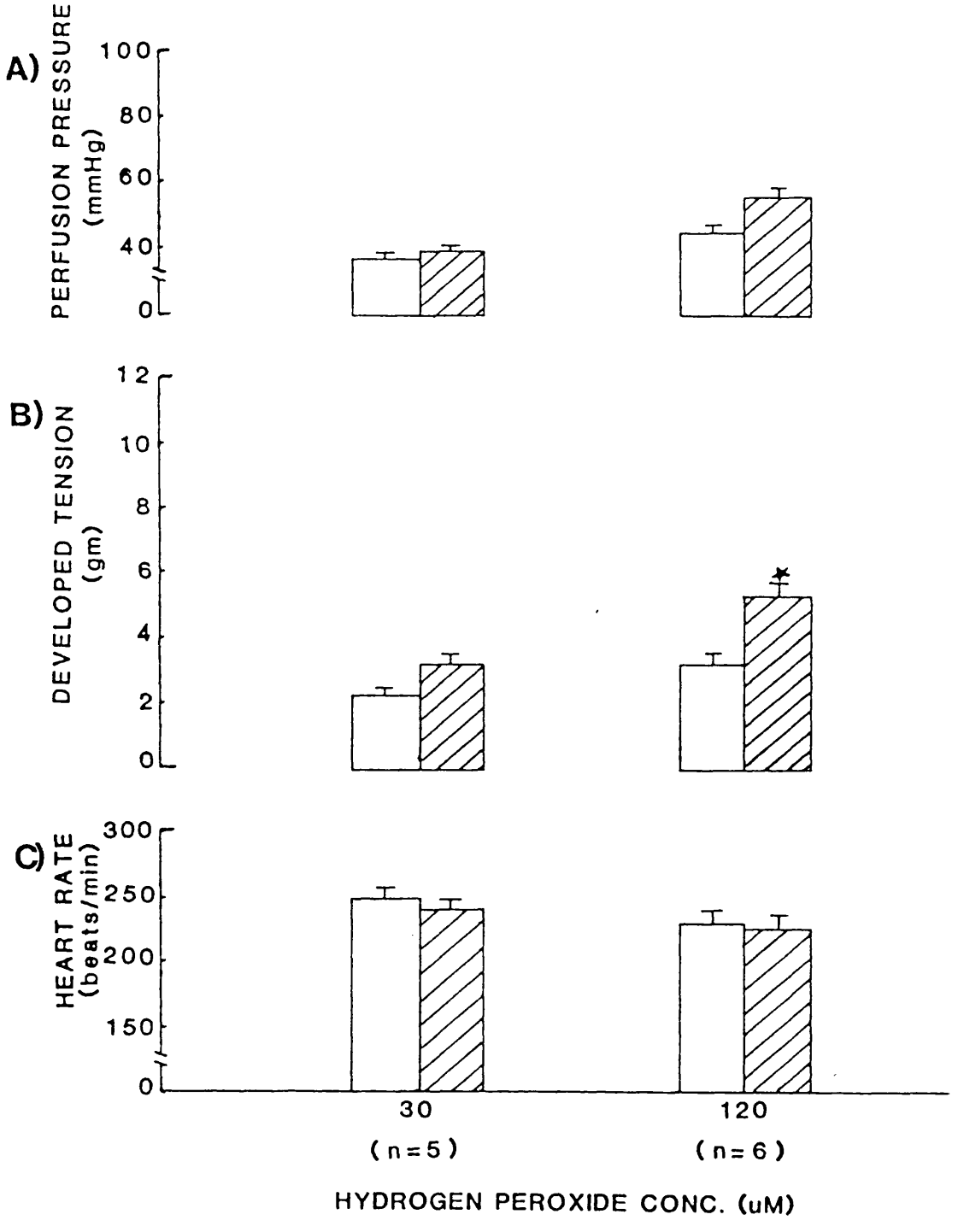


Fig. (53). Effects of hydrogen peroxide (3×10^{-5} and 12×10^{-5} M) on a) perfusion pressure, b) developed tension and c) heart rate in the isolated rat heart. Potassium 7.5 mM; magnesium 1.2 mM and calcium 1.2 mM.

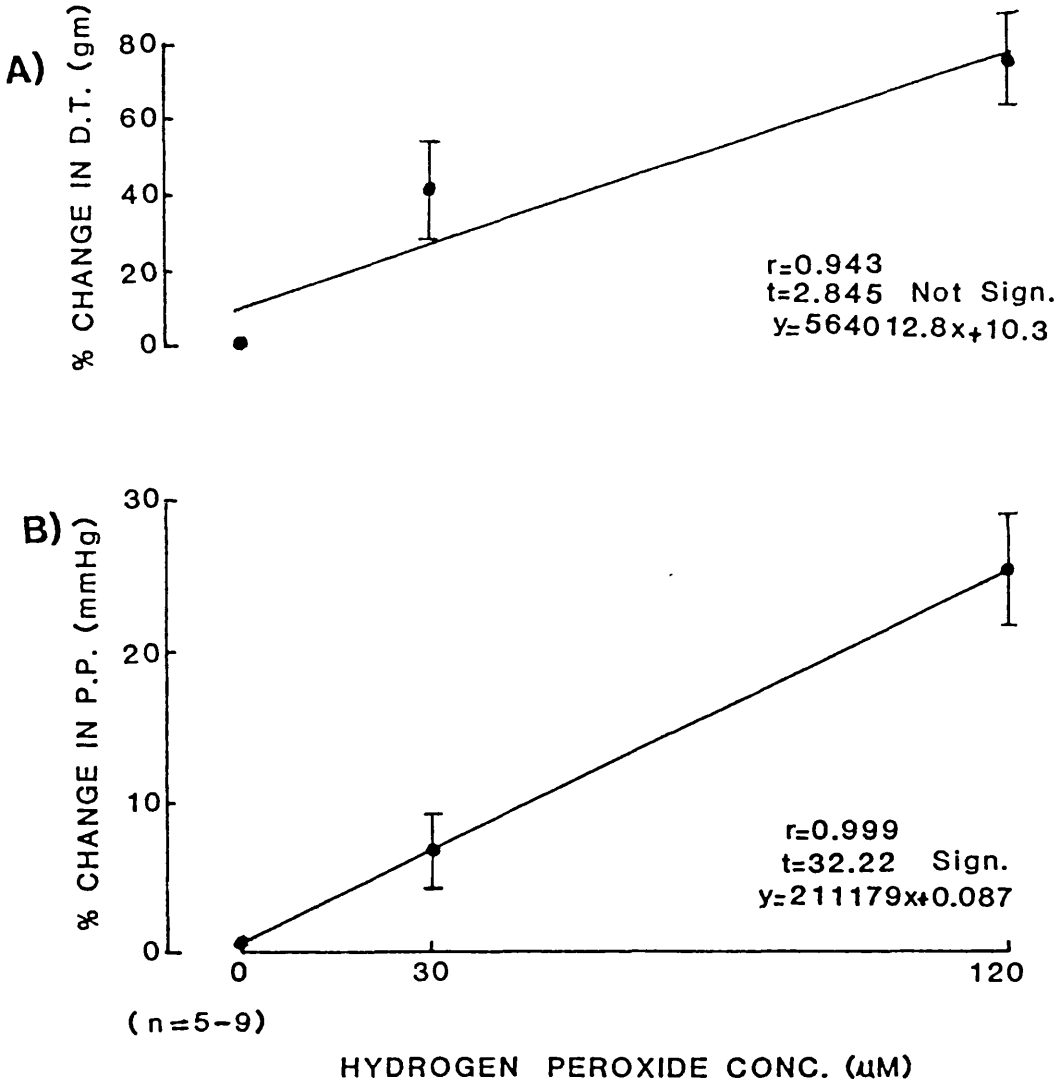


Fig. (54). Correlation between hydrogen peroxide (3×10^{-5} and 12×10^{-5} M) and a) change in developed tension % and b) change in perfusion pressure in the isolated rat heart. Potassium 7.5 mM.

that the protective effect of a higher potassium concentration may mask the effect of iron on the incidence of reperfusion arrhythmias. In Table (10) it can be seen that iron in the ferrous state increased the incidence of VT and VF while it had no effect on the number of PVCs or the onset and duration of VT and VF. The effect of ferrous ion was completely abolished by the addition of the iron chelator desferrioxamine to the perfusate. Neither ferric ion nor desferrioxamine alone had any significant effect on any of the criteria of arrhythmias studied (Table 10).

5.10: Effects of some free radical scavengers in the presence of ferrous ion on reperfusion induced arrhythmias

It was expected that the oxygen free radical produced by the Fenton reaction (Chapter 1.11) was the hydroxyl radical. Therefore specific scavengers for superoxide and hydroxyl radicals as well as catalase which prevents the formation of hydroxyl radicals by removal of hydrogen peroxide from the reaction medium were used to confirm what had been expected. As in Figs. (55) - (57) superoxide dismutase (10 units.ml^{-1}), catalase (100 and 300 units.ml^{-1}) and mannitol (2 x 10^{-2} M) reduced the incidence of VT. A significant reduction in the incidence of VF was produced by either catalase (300 units.ml^{-1}) or mannitol. There was no significant effect on the number of PVCs or the onset and duration of VT and VF.

5.11: Effects of some free radical scavengers when given after coronary ligation on reperfusion arrhythmias in presence of ferrous ion

Drugs were given 8 min after coronary artery ligation as mentioned before to make sure that the reduction in the incidence

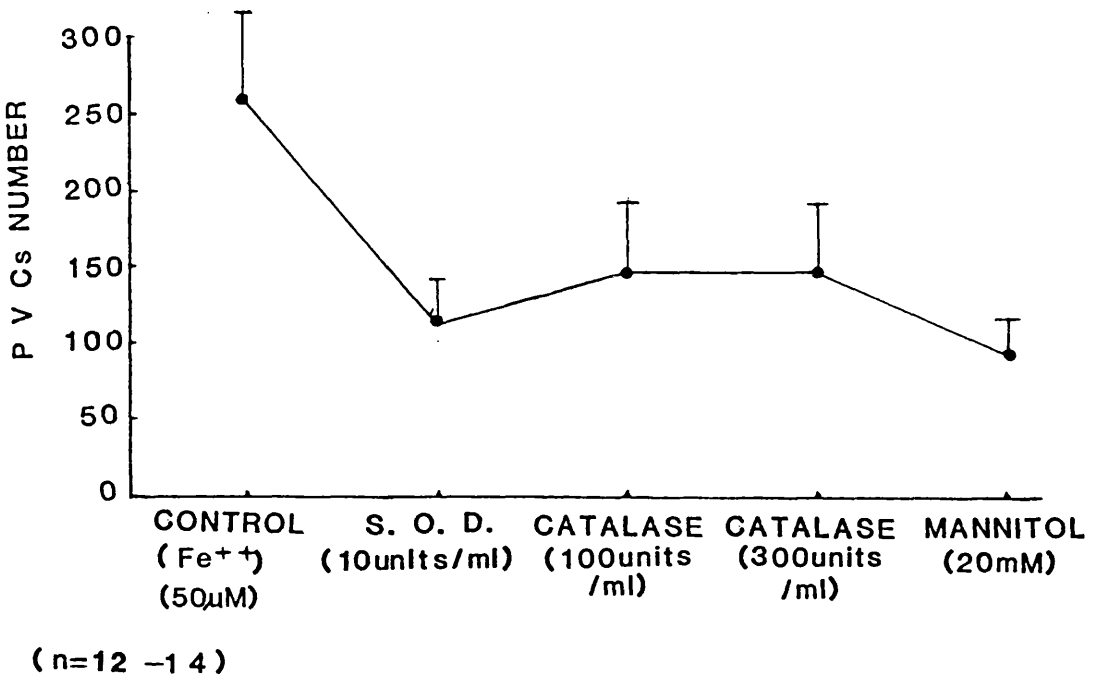


Fig. (55). Effect of superoxide dismutase (10 units.ml^{-1}), catalase (100 and 300 units. ml^{-1}) and mannitol (2×10^{-2} M) on the number of PVCs developed during reperfusion in presence of ferrous chloride (5×10^{-5} M).Potassium 5.9 mM; magnesium 1.2 mM and calcium 1.2 mM.

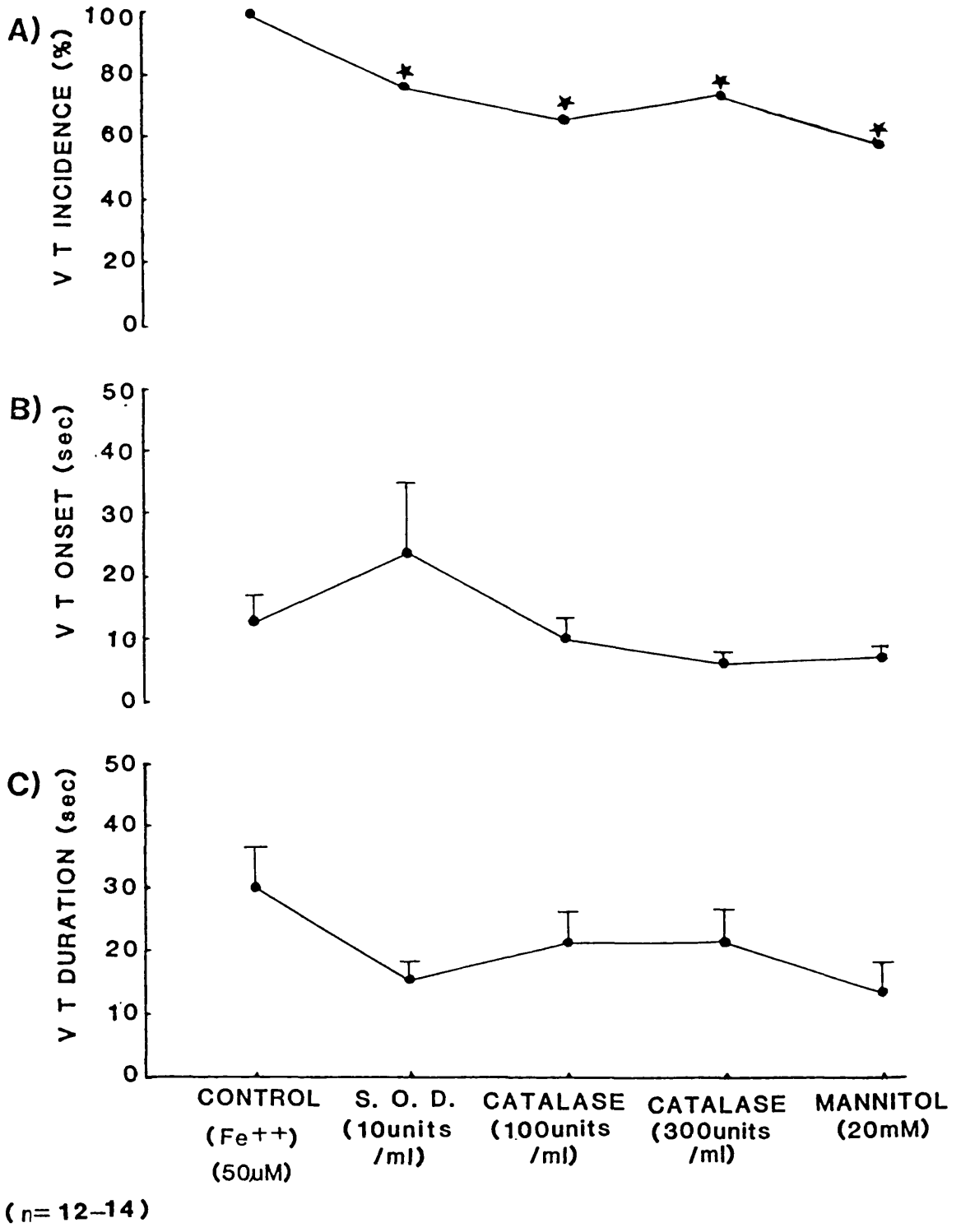


Fig. (56). Effects of superoxide dismutase (10 units.ml^{-1}), catalase (100 and 300 units.ml^{-1}) and mannitol (2 x 10^{-2} M) on a) the incidence, b) onset and c) duration of VT developed during reperfusion in presence of ferrous ion (5 x 10^{-5} M). Potassium 5.9 mM.

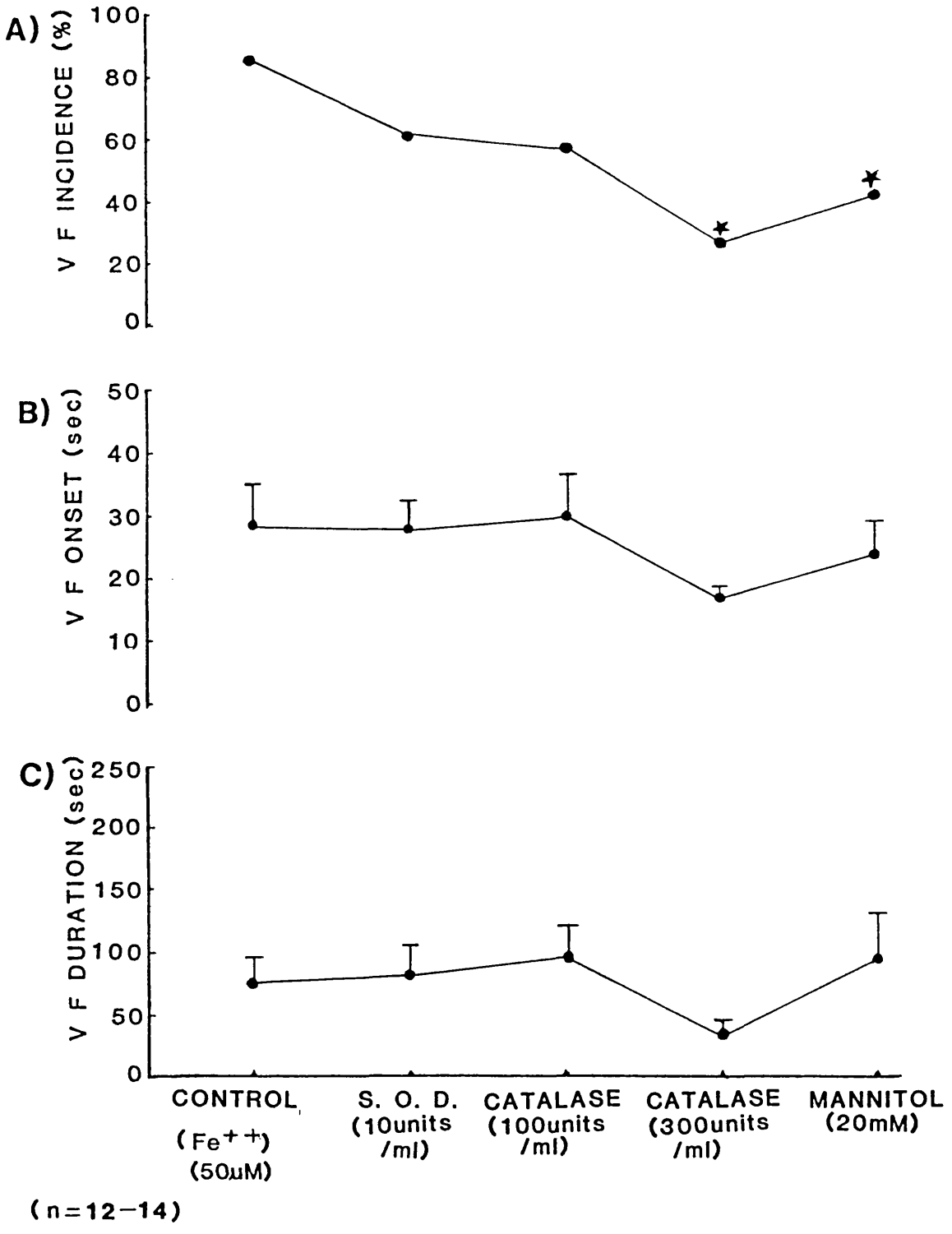


Fig. (57). Effects of superoxide dismutase (10 units.ml^{-1}), catalase (100 and 300 units.ml^{-1}) and mannitol (2x10^{-2} M) on a) the incidence, b) onset and c) duration of VF developed during reperfusion in presence of ferrous ion (5x10^{-5} M). Potassium 5.9 mM.

of reperfusion arrhythmias is not due to the reduction of the severity of the ischaemic damage which could indirectly reduce reperfusion arrhythmias. As in Figs. (58) - (60) histidine (5×10^{-3} M), catalase, (300 units.ml^{-1}), mannitol (2×10^{-2} M) and the mixture of superoxide dismutase plus catalase plus mannitol reduced the incidence of VT and VF. The reduction in the incidence of VT by mannitol was not significant. Only the mixture of superoxide dismutase plus catalase plus mannitol significantly reduced the number of PVCs (Fig. (58)), while, there was no significant effect on the onset and duration of VT (Fig. (59 b and c))and VF (Fig. (60 b and c)) except the reduction in the duration of VF produced by the mixture of superoxide dismutase,catalase and mannitol.

5.12: Effects of antagonists of free radical production on reperfusion induced arrhythmias

From Fig. (2) (Chapter 1.11) it can be seen that potential sources for the production of oxygen free radicals other than the mitochondria are: the autoxidation of noradrenaline to adrenochrome, the oxidation of xanthine by xanthine oxidase, and the conversion of PGG_2 to PGH_2. Therefore it was important to study the effect of blocking of each of these pathways on the incidence of reperfusion arrhythmias. 6-Hydroxydopamine which degenerates the adrenergic nerve endings was expected to prevent the production of superoxide radical from the autoxidation of noradrenaline as a result of prevention of noradrenaline release on reperfusion due to adrenergic nerve degeneration. The effect of nerve depletion by 6-hydroxydopamine was checked by showing block of the action of the indirect sympathomimetic agent tyramine. From Table (11) it can be seen

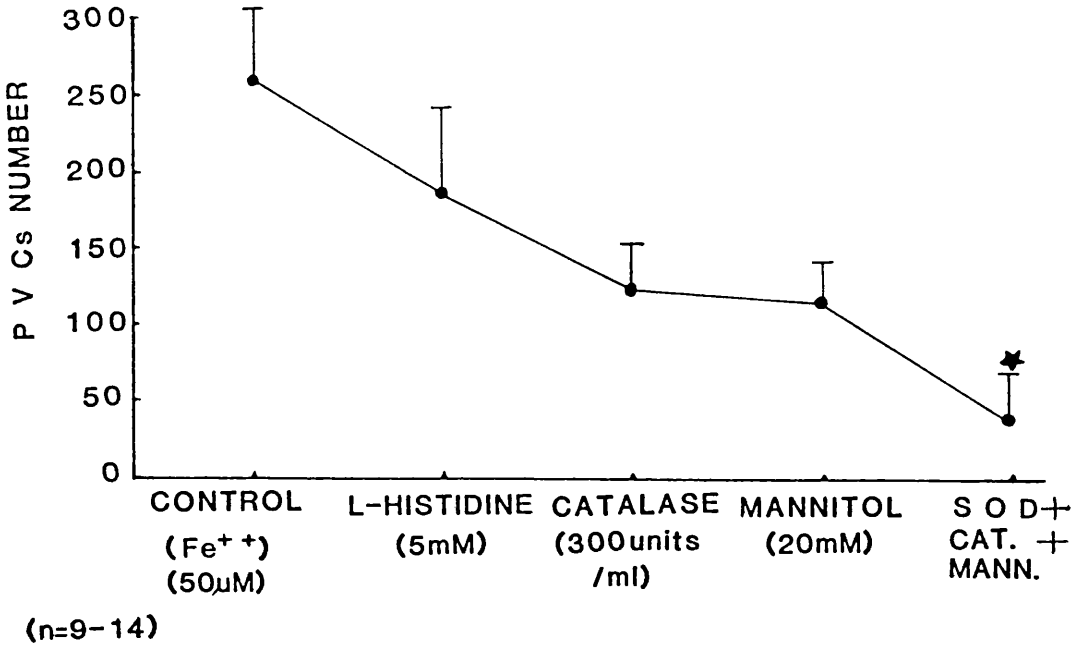


Fig. (58). Effect of L-histidine (5×10^{-3} M), catalase (300 units.ml^{-1}), mannitol (2×10^{-2} M) and the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) plus mannitol (2×10^{-2} M) when given 8 min after coronary artery ligation on the number of PVCs developed on reperfusion in the presence of ferrous ion. Potassium 5.9 mM; magnesium 1.2 mM and calcium 1.2 mM.

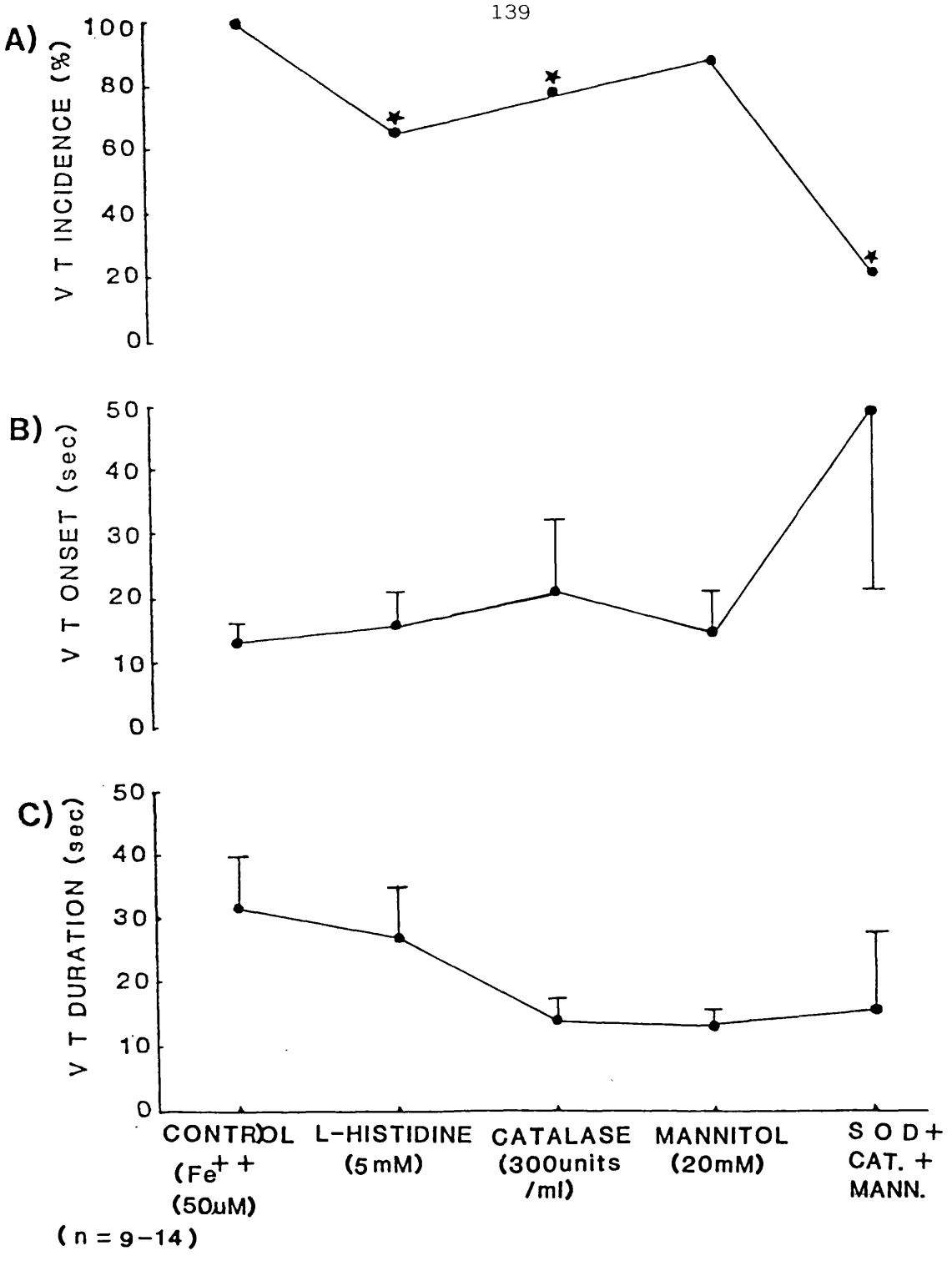


Fig. (59). Effects of L-histidine (5×10^{-3} M), catalase (300 units. ml^{-1}), mannitol (2×10^{-2} M) and the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) plus mannitol (2×10^{-2} M) when given 8 min after coronary artery ligation on a) the incidence,b) onset and c) duration of VT developed during reperfusion in the presence of ferrous ion (5×10^{-5}M). Potassium 5.9 mM.

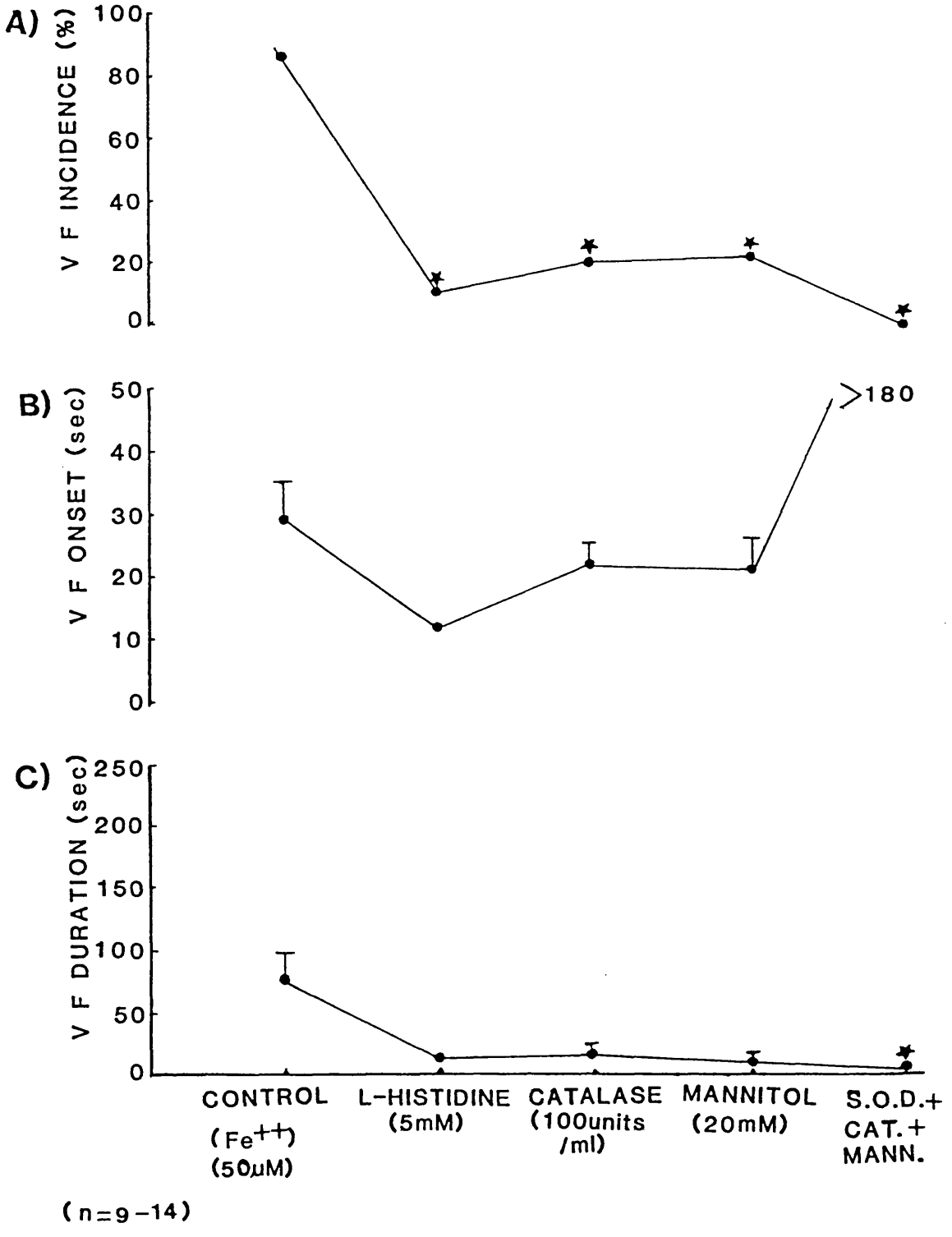


Fig. (60). Effects of L-histidine ($5x10^{-5}$M), catalase (300 units.ml^{-1}), mannitol ($2x10^{-2}$M) and the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) plus mannitol ($2x10^{-2}$M) when given 8 min after coronary artery ligation on a) the incidence, b) onset and c) duration of VF developed during reperfusion in the presence of ferrous ion ($5x10^{-5}$M).

Potassium 5.9 mM.

Table 11. Effects of 6-hydroxydopamine (50 mg.kg^{-1}, I.V., 18 hours before the experiment), ligation and reperfusion with oxygen free perfusate (95% N_2, 5% CO_2, 5 min before reperfusion) and reoxygenation after 10 min of global anoxia (95% N_2, 5% CO_2) on reperfusion induced arrhythmias in the isolated rat heart.

	n	PVCs/ 3 min	VT			VF		
			incidence (%)	onset (sec)	duration (sec)	incidence (%)	onset (sec)	duration (sec)
Control	21	142±32	86	12±4	13±3	91	21±5	99±16
6-Hydroxy-dopamine	9	178±55	78	9±2	29±7	33*	17±8	51±48
Lig + reper-fusion with O_2 free perfusate	10	113±44	60	12±5	14±5	0*	-*	-*
Global anoxia + reoxygenation	9	17±12	0*	-*	-*	0*	-*	-*

* $P < 0.05$
Control: 3.2 mM K^+, 1.2 mM Mg^{2+} and 1.2 mM Ca^{2+}

that 6-hydroxydopamine reduced the incidence of VF, while it had no significant effect on the number of PVCs, the incidence of VT or the onset and duration of VT and VF. On the other hand, dexamethasone, aspirin or indomethacin which block the synthesis of prostaglandins had no significant effect on the severity of reperfusion arrhythmias (Table 12). Similar to aspirin, allopurinol the competitive inhibitor of xanthine oxidase had no significant effect on the criteria of reperfusion induced arrhythmias when compared with its solvent dimethylacetamide (Table 12).

5.13: The role of oxygen in reperfusion induced arrhythmias

In all of the mechanisms expected to produce oxygen free radicals on reperfusion of ischaemic myocardium (Chapter 1.11) the availability of oxygen is essential. Therefore, it was of interest to study the effect of absence of oxygen from the reperfusate on reperfusion induced arrhythmias. As expected, oxygen free perfusate (5 min before reperfusion) completely prevented the incidence of reperfusion induced VF, although it had no significant effect on the number of PVCs or the incidence, onset and duration of VT (Table 11).

In order to find out if reperfusion induced arrhythmias are dependent on restoration of oxygen or flow or both, in a series of experiments, 10 min of global anoxia without myocardial ischaemia was induced. The results of these experiments are shown in Table (11). On reoxygenation following global anoxia VT or VF did not occur while there were only 17 ± 12 PVCs (n=9) compared with 142 ± 32 PVCs (n=21) developed on reperfusion of ischaemic

Table 12. Effects of dexamethasone (0.2 mg.kg^{-1}, I.V., 1 hour before the experiment), aspirin (10^{-4} M), indomethacin (5×10^{-5} M), dimethylacetamide (0.3 ml/L, control for allopurinol) and allopurinol (10^{-4} M) + dimethylacetamide (as solvent) on reperfusion induced arrhythmias in the isolated rat heart.

	n	PVCs/ 3 min	VT			VF		
			incidence (%)	onset (sec)	duration (sec)	incidence (%)	onset (sec)	duration (sec)
Control	21	142±32	86	12±4	13±3	91	21±5	99±16
Dexamethasone	9	100±23	78	6±1	10±2	78	14±1	49±23
Aspirin	9	171±51	78	11±1	27±8	67	25±2	145±10
Dimethyl-acetamide	10	202±109	90	14±5	17±9	70	13±2	35±20
Indomethacin	9	96±30	89	17±7	10±2	78	27±10	143±19
Allopurinol	11	161±29	100	10±1	12±2	55	15±2	70±31

Drugs were administered 5 min before coronary ligation except dexamethazone (1 hour before the experiment)

* $P < 0.05$. Control: 3.2 mM K^+, 1.2 mM Mg^{2+} and 1.2 mM Ca^{2+}.

myocardium. In Fig. (61) perfusion with oxygen free perfusate (for 5 min in the presence of myocardial ischaemia) and global anoxia (for 10 min) can be seen to cause vasodilator, negative inotropic and negative chronotropic effects in the isolated rat heart. From these results it can be seen that reperfusion induced arrhythmias are both flow and oxygen dependent and the role of restoration of flow is more predominant than restoration of oxygen.

5.14: Are free radicals produced during reperfusion?

The fact that free radical scavengers had a protective action against reperfusion arrhythmias suggests that free radicals are probably being produced during reperfusion and may contribute to the development of reperfusion arrhythmias. It was therefore of interest to try to obtain more direct evidence for the production of free radicals. In order to do this, hearts were perfused with ferricytochrome C throughout the ligation and reperfusion periods and the optical density of the perfusate after it passed through the heart was measured (see Methods: Chapter 2.3). From Fig. (62a) it can be seen that on reperfusion there was a significant transient increase in absorbance at 550 nm which returned to the pre-reperfusion value within 2 minutes. This transient increase in absorbance occurred at the same time as the reperfusion arrhythmias. As perfusate from ischaemic and non ischaemic mix during reperfusion it is not possible to determine the absolute value of reduced ferricytocrhome C coming from the ischaemic region. Therefore, the optical density readings have not been converted into concentrations of reduced ferricytochrome C. The increased absorbance at 550 nm in the presence of ferricytochrome C could be due to the reduction

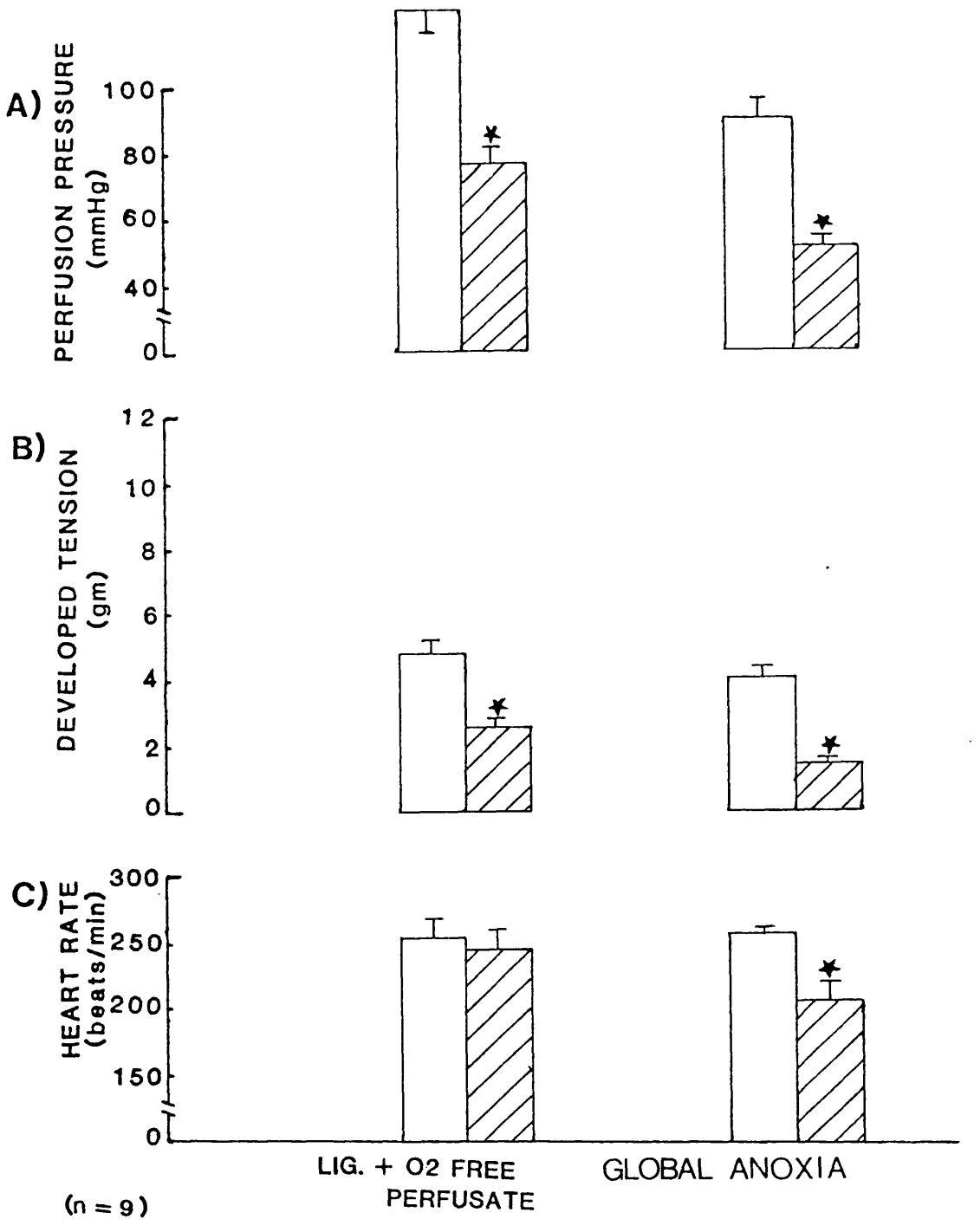


Fig. (61). Effects of perfusion with oxygen free perfusate (95% N_2, 5% CO_2) in the presence of regional myocardial ischaemia and global anoxia (95% N_2, 5% CO_2, for 10 min in the absence of myocardial ischaemia) on (a) perfusion pressure, (b) developed tension and (c) heart rate. Potassium 3.2 mM, magnesium 1.2 mM, and calcium 1.2 mM.

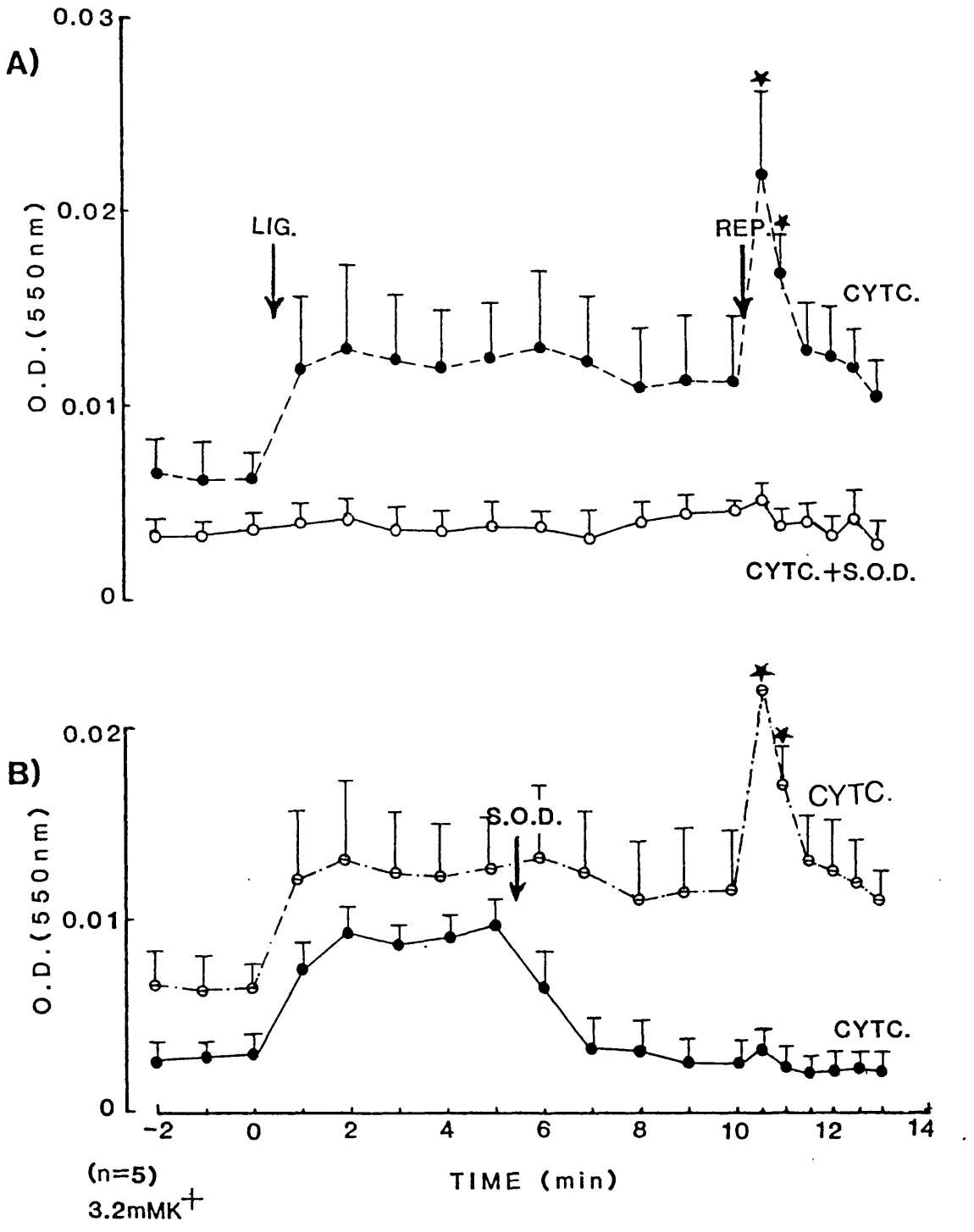


Fig. (62). Effect of coronary artery ligation and reperfusion on the optical density (550 nm) of perfusate from rat heart in the presence of (a) cytochrome C(CYTC , $5x10^{-5}M$) and cytochrome C plus superoxide dismutase (SOD, 10 units.ml^{-1}). In (b) superoxide dismutase was added at the arrow (5 min after coronary ligation). Potassium 3.2 mM. * Denotes significant difference from control preligation value($P<0.05$)

of ferricytochrome C by superoxide. However, there are other possible explanations for this change. It is possible that ferricytochrome C could be reduced by material other than superoxide; alternatively reperfusion and/or the development of arrhythmias could result in the release of some metabolite which absorbs at 550 nm. In order to try to eliminate these possibilities, the following experiments have been done:

(a) Hearts were perfused in the presence of ferricytochrome C and superoxide dismutase and the absorbance of the perfusate was recorded at 550 nm. Superoxide dismutase is specific scavenger for the superoxide ion and would be expected to prevent the reduction of ferricytochrome C if this was due to superoxide. From Fig. (62a) it can be seen that superoxide dismutase completely prevented the changes in optical density of the perfusate resulting from coronary artery ligation and reperfusion. Addition of superoxide dismutase during the coronary ligation period reduced optical density of the perfusate and prevented the increase seen when hearts were reperfused (Fig. 62b). The addition of boiled superoxide dismutase did not prevent the changes in optical density seen during ligation and reperfusion (n=3). These experiments provide evidence which suggests that ferricytochrome C is reduced by superoxide during coronary ligation and reperfusion.

(b) Hearts were reperfused in the absence of ferricytochrome C to see if reperfusion releases any material which absorbs at 550 nm. In these experiments there was no change in the optical density at 550 nm during coronary artery ligation and reperfusion (n=5). This suggests that the changes in

optical density seen in the presence of ferricytochrome C were due to the reduction of ferricytochrome C and not to the release of some other material which absorbs at 550 nm.

(c) Hearts were made to develop VF in the absence of coronary ligation and reperfusion to see if VF releases any material which absorbs at 550 nm. Ventricular fibrillation was induced by perfusing hearts with an arrhythmogenic solution (see Methods, Chapter 2) which produced VF within 255 ± 10 sec (n=5). The onset of VF was not associated with any change in the optical density of the perfusate at 550 nm. This provides evidence that the increase in optical density at 550 nm in the presence of ferricytochrome C during reperfusion was due to the reduction of ferricytochrome C and was not due to VF releasing material which absorbs at this wavelength.

In order to find out the role of oxygen in production of free radicals, hearts were perfused with ferricytochrome C throughout the ligation and reperfusion period. Oxygen free perfusate was used (5 min after coronary ligation till 3 min after reperfusion). As in Fig. (63a) elimination of oxygen completely prevented the transient increase in absorbance at 550 nm expected to be produced on reperfusion.

In order to confirm that the protective effect of 6-hydroxy-dopamine may be produced by prevention of superoxide production from autoxidation of noradrenaline the change in optical density due to reduction of ferricytochrome C throughout the coronary ligation and reperfusion periods was measured in 6-hydroxydopamine

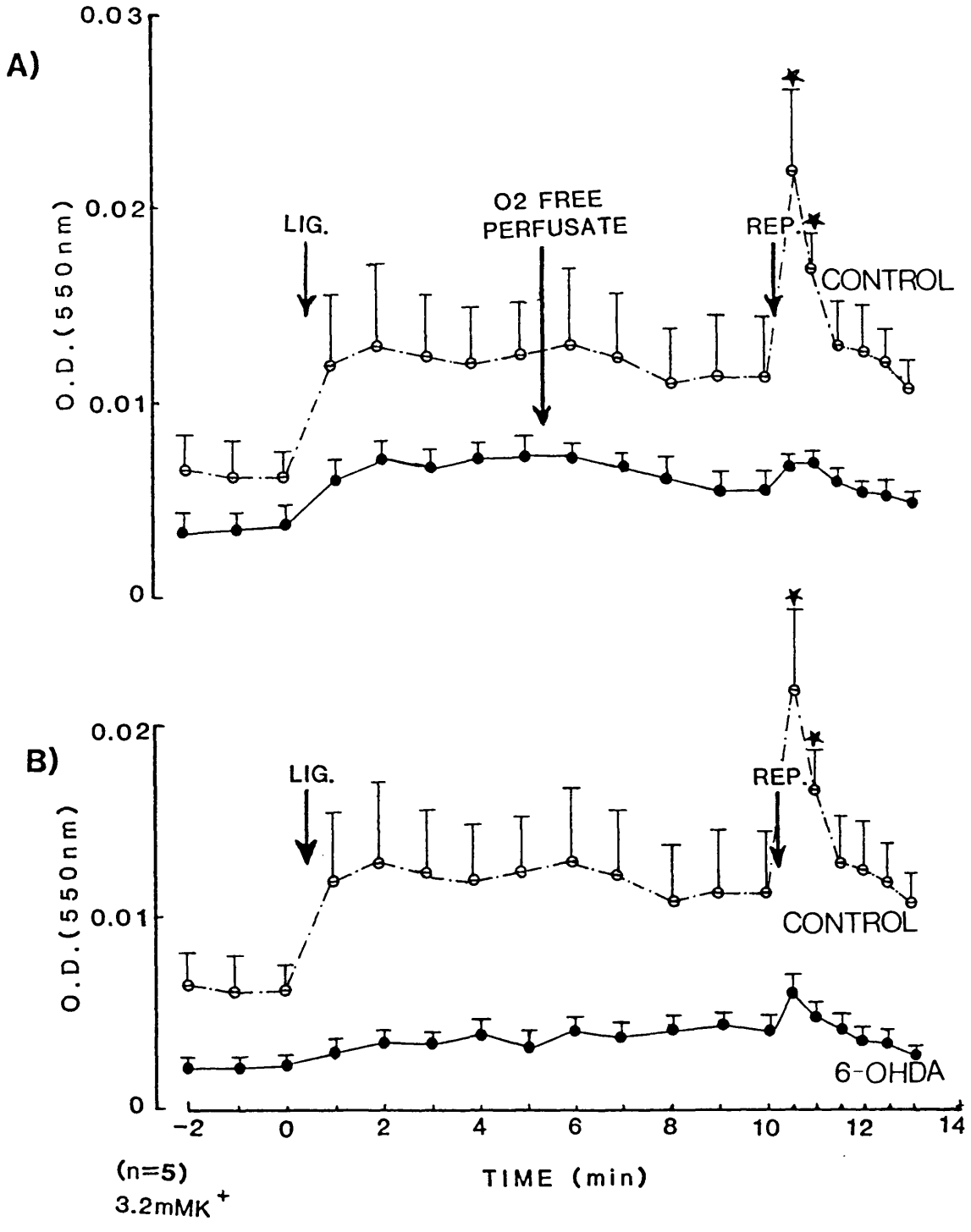


Fig. (63). Effect of coronary artery ligation and reperfusion on the optical density (550 nm) of perfusate from rat heart in the presence of (a) oxygen free perfusate (95% N_2 and 5% CO_2) used at the arrow (5 min after coronary ligation). In (b) animals used were injected with 6-hydroxydopamine (50 $mg.kg^{-1}$) I.V., 18 hours before study.

treated rats. From Fig. (63b) it can be seen that 6-hydroxydopamine prevented the increased absorbance at 550 nm in the presence of ferricytochrome C throughout coronary ligation and reperfusion periods.

From Fig. (64a) it can be seen that allopurinol approximately had no effect on the change in optical density when compared with the effect produced by its solvent dimethylacetamide which reduced non significantly the increase in absorbance at 550 nm produced during coronary ligation and reperfusion in control group. Although this effect of dimethylacetamide, the transient increase in absorbance at 550 nm produced on reperfusion in the presence of dimethylacetamide was still significantly different from the pre-ligation values.

When indomethacin was added to the perfusate in the presence of ferricytochrome C, the transient increase in the absorbance at 550 nm was still significantly different from the pre-ligation values although it was significantly lower than that increase in absorbance at 550 nm in the absence of indomethacin (Fig. (64b)). This may indicate that free radical generation through arachidonic acid metabolism is possible.

5.15: Protein release from the heart during myocardial ischaemia and reperfusion

As a result of oxygen free radical generation, peroxidation of cell membrane which will lead to loss of the integrity of the cell membrane and release of intracellular protein and enzymes

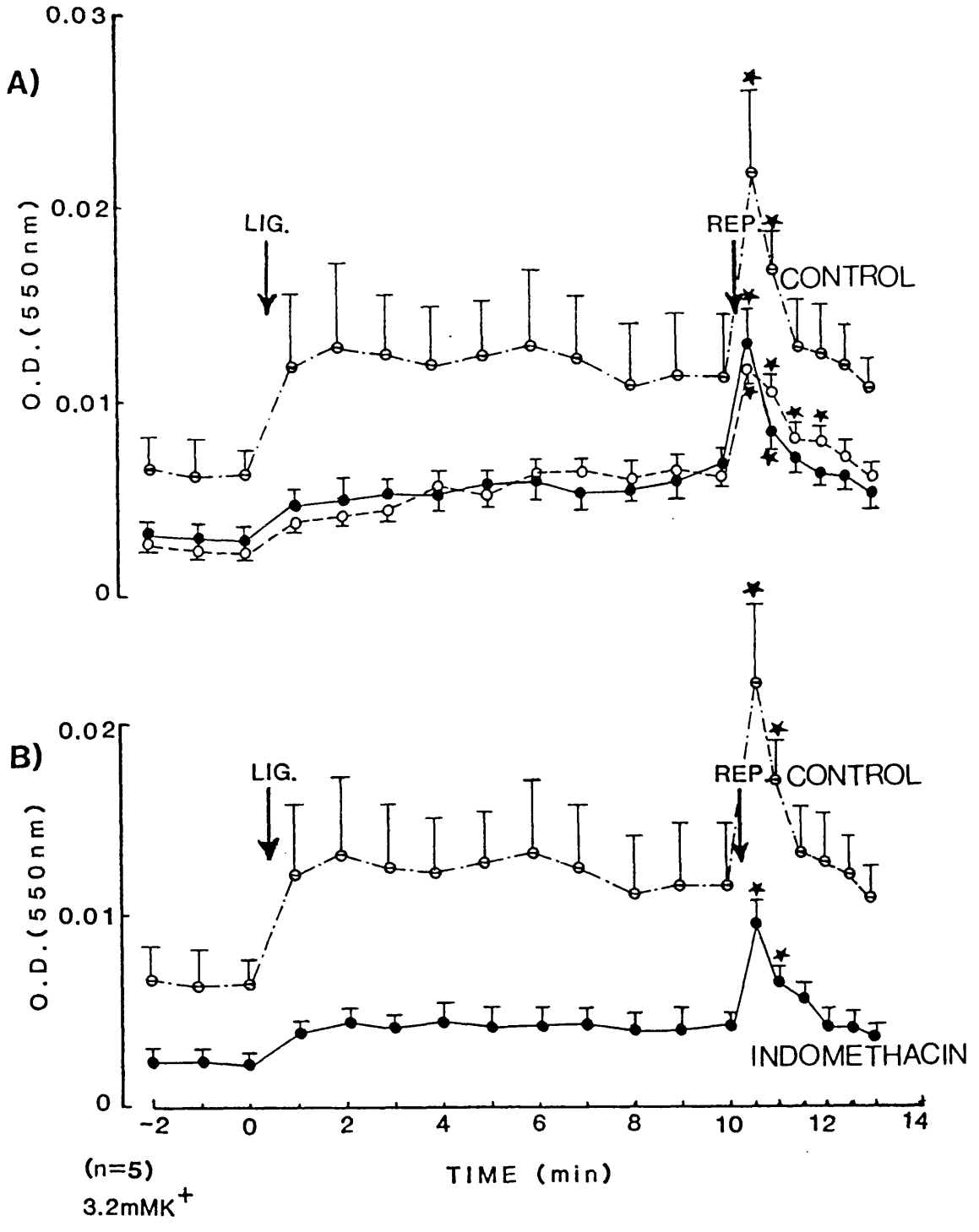


Fig. (64). Effect of coronary artery ligation and reperfusion on the optical density (550 nm) of perfusate from rat heart in the presence of (a) allopurinol (10^{-4}M) plus dimethylacetamide as a solvent (-o--o-) and dimethylacetamide alone (0.3ml.L^{-1}, —●—●—). In (b) indomethacin ($5x10^{-5}$M) was added 5 min before coronary ligation. * refer to significant difference compared with preligation values.

was expected. Therefore, it was worthwhile to try to determine the protein concentration in the perfusate and malondialdehyde, the lipid peroxidation product in heart tissues after coronary artery ligation and reperfusion. In Table (13a) it can be seen that after 10 min of coronary ligation there was no significant increase in perfusate protein concentration, while, on reperfusion protein concentration significantly increased from 15.6 ± 1.1 µg/ml to 19.4 ± 0.7 µg/ml after one minute and to 20.6 ± 1.8 µg/ml after two minutes. When the coronary ligation period was increased to 30 min (Table 13b) there was no significant change in protein concentration during the first 20 min but it has been significantly increased from 14 ± 0.5 µg/ml (before coronary ligation) to 23.5 ± 2.4 µg/ml after 30 min of coronary ligation. The increase in protein concentration was also significant during the first two minutes after ligation, it came down to the control value after 3 min.

5.16: Lipid peroxidation induced by 10 min ischaemia and reperfusion

For determination of malondialdehyde, the lipid peroxidation product a group of 9 rats were made deficient in vitamin E by feeding with a vitamin E deficient diet for 8 weeks in order to potentiate lipid peroxidation by free radicals as vitamin E is part of the normal defense mechanism. Malondialdehyde was measured in both the ischaemic and nonischaemic tissues. There was no significant difference in malondialdehyde concentration (nMoles.g^{-1} wet weight) between the ischaemic (13.4 ± 1.2) and nonischaemic (10.1 ± 0.6) values estimated in the vitamin E deficient group. Similar results were obtained in a control group (n=9), the malondialdehyde concentration (nMoles.g^{-1} wet weight) in the ischaemic tissue was

Table 13. The concentration of protein ($\mu g.ml^{-1}$) in the perfusate measured during (a) 10 min of coronary artery ligation and reperfusion. (b) 30 min of coronary artery ligation and reperfusion. Results are presented as mean ± S.E.M. (n=5). * denotes significant difference from the preligation value ($P < 0.05$). Potassium 3.2 mM. CAL: Coronary artery ligation.

	Protein concentration ($\mu g.ml^{-1}$)						
	Pre-ligation value	10 min after CAL	20 min after CAL	30 min after CAL	1 min after reper-fusion	2 min after reper-fusion	3 min after reperfusion
a) 10 min of ischaemia and reperfusion	14.15±5.15	15.62 ± 1.11			19.35*± 0.736	20.62* ± 1.81	19.6 ± 2.15
b) 30 min of ischaemia and reperfusion	14.05±0.47	16.96 ± 2.09	19.24 ± 2.44	23.54*± 2.37	26.56*± 2.28	23.46*± 1.44	14.04 ± 3.26

13.1 ± 1.0 compared with 9.4 ± 0.6 in the nonischaemic tissue. Serum vitamin E levels were 20.1 ± 0.7 $\mu Moles.ml^{-1}$ in the control group compared with 11.0 ± 0.6 $\mu Moles.ml^{-1}$ in the deficient group. Although vitamin E was significantly reduced in the serum of rats expected to be deficient in vitamin E , it was still more than half of the control value. This could explain why malondialdehyde was not significantly higher in the deficient group compared with the control group. Similar to malondialdehyde, when lactate was determined in the perfusate there was no significant difference between lactate concentration ($\mu Moles.ml^{-1}.g^{-1}$ wet weight) values in control group and vitamin E deficient group(Table 14).

5.17: Effects of some antagonists of the arachidonic acid metabolism on reperfusion induced arrhythmias

oxygen free radicals can be produced during myocardial ischaemia as a result of arachidonic acid metabolism (Chapter 1.11). This would indirectly be expected to affect the development of reperfusion arrhythmias. Furthermore, most of arachidonic acid metabolites are vasoactive agents and this will in turn affect the severity of ischaemic damage and the rate of reperfusion leading to a decrease or increase in the incidence of reperfusion arrhythmias. For these two reasons it was worthwhile to study the effect of arachidonic acid metabolites by studying the effect of the blockers of their synthesis on reperfusion induced arrhythmias. From Tables (12) and (15) it can be seen that dexamethasone, aspirin, indomethacin, ZK 36374 (10^{-10} M), dazoxiben (10^{-6} M) and N-butyl-imidazole (10^{-6} M) had no effect on the number of PVCs or the incidence, onset and duration of VT and VF. The higher concentrations

Table 14. Lactate concentration ($\mu Moles.ml^{-1}.g^{-1}$ wet weight) in the perfusate (a) control group (n=9) and (b) vitamin E deficient group (n=9). Results are presented as mean ± S.E.M. * Denotes significant difference from the pre-ligation value ($P < 0.05$).

	Lactate concentration ($\mu Moles.ml^{-1}.g^{-1}$ wet weight)				
	Preligation	10 min after ligation	1 min after reperfusion	2 min after reperfusion	3 min after reperfusion
a) Control group	0.199±0.04	0.250±0.07	0.406±0.07	0.287±0.05	0.216±0.04
b) Vit. E deficient group	0.092±0.02	0.145±0.02	0.321±0.05	0.185±0.03	0.140±0.03

Table 15. Effects of N-butylimidazole (10^{-6} M), BW 755C (10 $\mu g.ml^{-1}$), Z.K. 36374 (10^{-10} and 10^{-9} M) and dazoxiben (10^{-6} M and 10^{-5} M) on reperfusion induced arrhythmias in the isolated rat heart

	n	PVCs/ 3 min	VT			VF		
			incidence (%)	onset (sec)	duration (sec)	incidence (%)	onset (sec)	duration (sec)
Control	21	142±32	86	12±4	13±3	91	21±5	99±16
N-butylimidazole (10^{-6}M)	9	94±18	100	15±4	8 ±1	67	19±2	109±33
BW 755C (10 $\mu g.ml^{-1}$)	9	96 ± 23	89	19±5	11±3	44*	24±7	46±34
Z.K. 36374								
(a) 10^{-10}M	6	221±84	83	20±5	30±15	83	31±5	23±15
(b) 10^{-9} M	11	93±32	82	23±5	13±4	45*	25±3	100±31
Dazoxiben								
(a) 10^{-6} M	9	122±26	89	12±3	13±3	67	38±15	112±28
(b) 10^{-5} M	9	91±16	89	14±3	10±2	44*	23±4	120±36

Drugs were administered 5 min before coronary ligation. * $P < 0.05$.

Control: 3.2 mM K^+, 1.2 mM Mg^{2+} and 1.2 mM Ca^{2+}.

of ZK 36374 (10^{-9} M) and dazoxiben (10^{-5} M) as well as BW 755C (10 $\mu g.ml^{-1}$) reduced the incidence of VF while having no effect on the number of PVCs, incidence of VT or on the onset and duration of VT and VF (Table 15). Indomethacin reduced perfusion pressure (Fig. (65)) while the rest of these drugs had no significant effect on perfusion pressure, developed tension or heart rate (Figs. (65) and (66)).

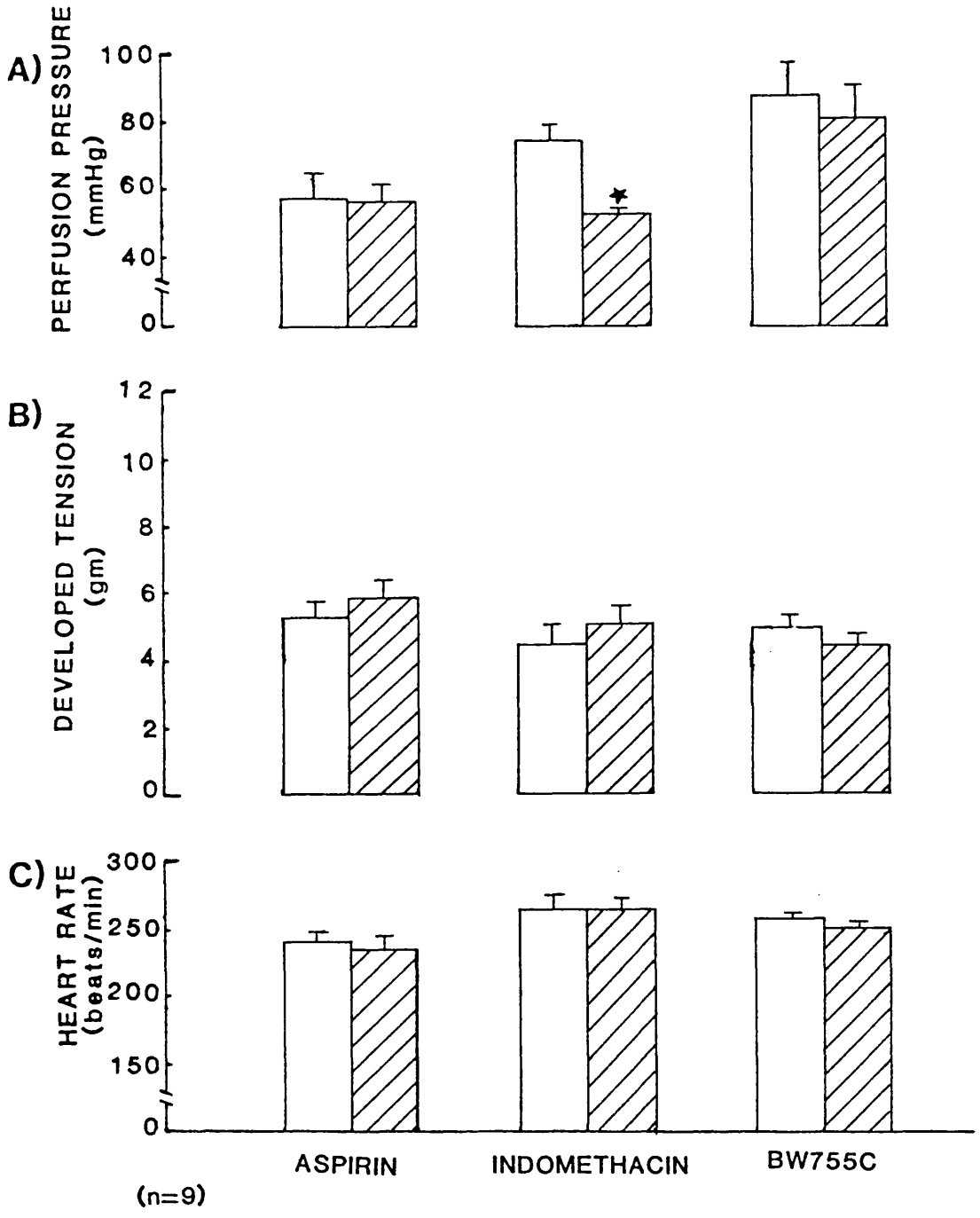


Fig. (65). Effects of aspirin (10^{-4} M), indomethacin (5 x 10^{-5} M) and BW 755C (10 $\mu g.ml^{-1}$) on (a) perfusion pressure, (b) developed tension and (c) heart rate in the isolated rat heart. Potassium 3.2 mM, magnesium 1.2 mM, and calcium 1.2 mM.

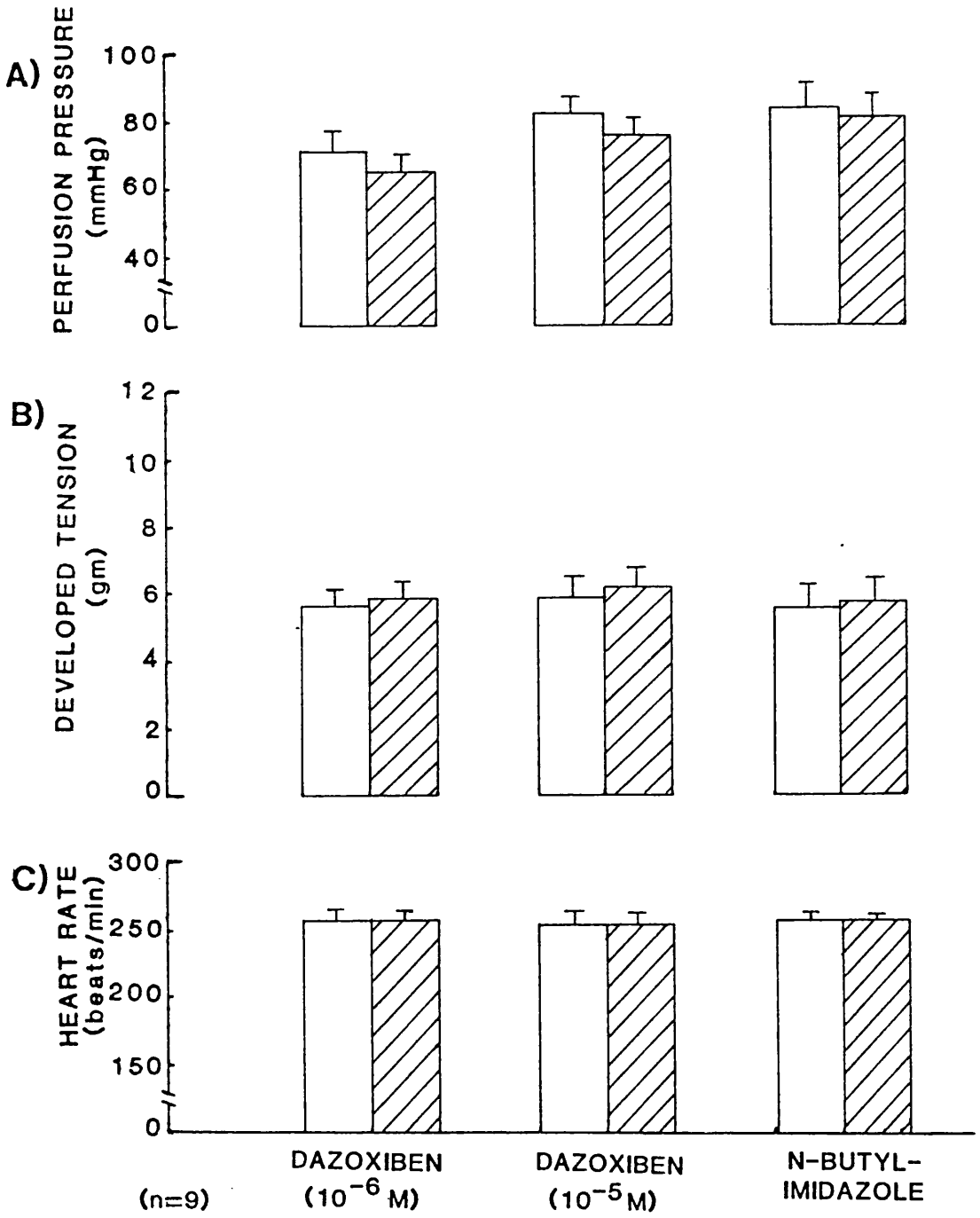


Fig. (66). Effects of dazoxiben (10^{-6} and 10^{-5} M) and N-butyl-imidazole (10^{-6} M) on (a) perfusion pressure, (b) developed tension and(c) heart rate in the isolated rat heart. Potassium 3.2 mM, magnesium 1.2 mM and calcium 1.2 mM.

Section B: Discussion

Oxygen free radicals have been implicated in the peroxidation of lipid in cell membranes (Meerson *et al.*, 1982; Koster *et al.*, 1985). This will alter the ionic permeability of the sarcolemma and the distribution of Na^+, K^+ and Ca^{2+} between the extra- and intracellular compartments. Oxygen free radicals have also been implicated in the myocardial damage induced by isoprenaline in rats (Singal *et al* ., 1983) and by coronary artery ligation in dogs (Rao *et al.*, 1983). In both situations treatment aimed at preventing or reducing the production of oxygen free radicals was shown to have a myocardial protective action. It has been suggested that free radicals might be involved in initiating isoprenaline-induced rhythm disturbances in the rat (Singal *et al.*, 1983). Adriamycin, the anticancer drug, has been reported to induce free radical mediated lipid peroxidation in mouse heart (Mimnaugh *et al.*, 1983) and this could explain why this drug produces arrhythmias in patients. However, there is little direct evidence linking free radicals to the development of reperfusion induced arrhythmias. In the present study drugs which have been reported to remove oxygen free radicals from the reaction media protected against reperfusion induced arrhythmias in the isolated rat heart.

Under normal conditions, biological tissues are protected against the damaging effect of oxygen free radicals by the bodies defense mechanisms afforded by the various antioxidant systems in the body (Dormandy, 1978). It is only when there is excessive

production of oxygen free radicals or when the normal defense mechanisms are not functioning properly that free radicals accumulate and exert their damaging effects (Meerson *et al* ., 1982). The release of catecholamines during reperfusion (Rochette *et al*., 1980) and their subsequent auto-oxidation could produce oxygen free radicals (Burton *et al*., 1984). Moreover, oxygen free radicals can be produced during myocardial ischaemia during the conversion of prostaglandin G_2 to prostaglandin H_2 (Rowe *et al*., 1983; Hertz and Cloarc, 1984; Van der Vusse and Reneman, 1985). which is expected to affect reperfusion arrhythmias. In addition, xanthine oxidase could be another source for superoxide radical production (Burton *et al*., 1984). In the present study, it has been shown that inhibition of xanthine oxidase or prostaglandin synthesis had no significant effect on the severity of reperfusion arrhythmias although they markedly decreased the production of superoxide free radicals (Fig. (64)). An explanation of this could be due to the fact that the beneficial effect of cyclo-oxygenase pathway inhibition has been masked by stimulation of the lipoxygenase pathway. From Fig. (64a) it can be seen that allopurinol, the inhibitor of xanthine oxidase enzyme had no effect on superoxide production but the effect is due to the solvent used (dimethylacetamide). The lack of any protective effect of allopurinol on reperfusion arrhythmias is in agreement with the recent findings of Reimer and Jennings (1985) who have shown that allopurinol has no beneficial effect on reperfusion induced myocardial infarction in the anaesthetized dog. The protective effect of 6-hydroxydopamine administration coupled with the prevention of the reduction of ferricytochrome C on reperfusion support the

concept that catecholamines released on reperfusion (Rochette *et al.*, 1980) could produce oxygen free radicals which are the risk factor. In recent study in the isolated rat heart physiological metabolites and the non-physiological metabolite adrenochrome have not been shown to be responsible for catecholamine-induced myocardial cell damage (Wheatley *et al.*, 1985). Similarly, the protective effect of the elimination of oxygen from the reperfusate in the present study confirms the necessity to oxygen for the generation of free radicals by all sources of production of free radicals.

Membrane damage due to membrane lipid peroxidation could be one of the causes of the arrhythmias seen during coronary reperfusion. Free radicals have been shown to inhibit calcium uptake by the sarcoplasmic reticulum (Hess *et al.*, 1981). This coupled with the influx of calcium through the damaged sarcolemma would cause an increase in intracellular free calcium which can in turn initiate arrhythmias (Woodward, 1981; Clusin *et al.*, 1982). In human neutrophils (Simchowitz and Spilberg, 1979) increased intracellular calcium levels increase superoxide production. If calcium has a similar effect on superoxide production in the myocardium, it could be argued that superoxide production would be enh anced during myocardial ischaemia and reperfusion as intracellular levels of calcium do increase during these pathological events.

From the results with oxygen free radical scavengers in the absence of ferrous ion it is not possible to say which if any of the free radicals is the most important in contributing to

arrhythmogenesis. The present study has only provided evidence from experiments with ferricytochrome C for the existence of the superoxide anion. However, the fact that the combination of catalase and mannitol produced a significant reduction in the incidence of VF would indicate that hydrogen peroxide and the hydroxyl free radical may also be produced during reperfusion. Superoxide dismutase, glutathione, catalase and ascorbic acid will reduce the level of superoxide and/or hydrogen peroxide (Halliwell, 1981). The lowering of the levels of these toxic metabolites may explain the antiarrhythmic actions of these compounds. Alternatively, they may protect by reducing the formation of hydroxyl free radicals, as superoxide and hydrogen peroxide are both substrates for hydroxyl radical production in the presence of transition metals (Halliwell and Gutteridge, 1984). When ferrous ion was used in the present study it markedly increased the severity of reperfusion arrhythmias. This could be due to the enhanced production of hydroxyl radicals via the Fenton reaction. From the present investigation, it can be seen that in the absence of ferrous ion superoxide dismutase protected against reperfusion arrhythmias while either catalase or mannitol failed to protect. In the presence of ferrous ion where hydroxyl radicals are expected to predominate over the other free radicals, superoxide dismutase (which was expected to increase arrhythmias) had no significant effect on reperfusion arrhythmias while either catalase which prevents the formation of hydroxyl radicals or mannitol which reacts with this radical did protect. This suggests that in the absence of exogenous ferrous ion the predominant free radical is superoxide although the other free radicals could exist. In the presence of traces of transition metals the rate of production of the hydroxyl radical is enhanced

(Halliwell and Gutteridge, 1984) which may lead to predomination of the hydroxyl radical over the other free radicals being produced during reperfusion.

One of the problems with the interpretation of all of the results in this chapter is that superoxide dismutase, catalase, glutathione and mannitol would be expected to remain in the extracellular compartment. This would be expected to greatly reduce or abolish their activity as they would not have access to free radicals which are generated intracellularly. In a study with erythrocyte ghosts, Lynch and Fridovich (1978) have shown that superoxide dismutase in the external medium protected against superoxide radicals generated inside erythrocyte ghosts and they proposed that in the absence of any superoxide radical scavenger in the external medium, internal and external superoxide radicals are in equilibrium through an anion carrier mechanism. Thus the destruction of extracellular superoxide radical by superoxide dismutase would decrease the steady-state level of intracellular superoxide anion. This proposal may explain the activity of these scavengers in spite of their very high molecular weight which might be expected to keep them in the extracellular compartment. Another problem is that by which mechanism is mannitol acting: hyperosmolarity or hydroxyl scavenging? In the present study hyperosmolar solution of glucose has been used as control to mannitol (not mentioned in this thesis) but it had no effect on reperfusion induced arrhythmias. This would suggest that any beneficial effect of mannitol on reperfusion arrhythmias might not be due to its osmotic properties. In agreement with this finding, Magovern

et al. (1984) using the isolated rabbit heart showed that mannitol markedly reduced myocardial oedaema formation during reperfusion compared with hearts treated with hyperosmolar solution of glucose.

Most of the results in this chapter except Figs. (50) - (52) and (58) - (60) were obtained by adding drugs to the perfusate 5 minutes before the onset of coronary ligation. This regime of drug administration could reduce the extent of ischaemic damage as free radicals are produced during ischaemia (Rao *et al.*, 1983). This in turn could indirectly reduce the severity of reperfusion arrhythmias. From Fig. (50) - (52) and (58) - (60) it is clear that the late administration of free radical scavengers 2 min before reperfusion still reduced the incidence of ventricular fibrillation. This implies that these drugs have a direct beneficial effect against reperfusion induced arrhythmias. If the effect of these free radical scavengers on reperfusion induced arrhythmias when given 5 min before ligation is compared with their effect when given 2 min before reperfusion it can be seen that they had a greater antiarrhythmic action when given before the onset of coronary artery ligation. Therefore, these results probably reflect a combination of antiischaemic and antiarrhythmic actions.

When experiments were carried out with ferricytochrome C there was an increased reduction of ferricytochrome C during the first minute of reperfusion which was prevented by superoxide dismutase. This provides evidence for the production of superoxide radical during the first minute of reperfusion. There are at least two possible explanations for the peak of reduced ferricytochrome

C which is seen during reperfusion. One explanation is that sudden reperfusion of ischaemic tissue enhances superoxide radicals production. This could occur as already discussed, if the sudden influx of oxygen into the ischaemic tissue produced excess superoxide which overcame the endogenous antioxidant protective mechanisms in the heart. A second possibility is that this peak of reduced ferricytochrome C may simply represent the washout of reduced ferricytochrome C which has accumulated during the ischaemic period. The release of catecholamines during reperfusion has also been attributed to this process (Rochette *et al*., 1980). In the dog, free radicals are produced during ischaemia (Rao *et al*., 1983) and in the present experiments there was a tendency towards an increased reduction of ferricytochrome C during the ischaemic period. Therefore this second possibility cannot be dismissed.

In the present study when either oxygen was eliminated during reperfusion or when global anoxia was followed by reoxygenation the development of VF was completely prevented. Elimination of oxygen during reperfusion had no effect on the incidence of VT. This suggests that although reperfusion arrhythmias are dependent on restoration of both flow and oxygen, restoration of flow seems to play more important role in arrhythmogenesis than reoxygenation. The present results are consistent with the findings by Carbonin *et al*. (1981) in the isolated rat heart that both sufficient degree of myocardial damage provoked by the preceding ischaemia and the presence of oxygen during reperfusion are necessary for the development of reperfusion induced tachyarrhythmias. Moreover, Petropoulos and Meijne (1964) had shown that reperfusion with

venous blood or anoxic saline had no effect on the incidence of reperfusion induced ventricular fibrillation in the dog suggesting the predominance of restoration of flow over the reoxygenation process. When experiments were carried out in a group of animals deficient in vitamin E (a member of the endogenous protective mechanisms in the biological tissue, Trush *et al.*, 1982; Frank, 1983) there was no significant increase in tissue damage and lipid peroxidation. This could be due to insufficient vitamin E depletion, as vitamin E was still present in the serum of rats although it has been decreased to about one half of the control value. This suggests that 8 weeks of vitamin E depletion is not sufficient. In a similar study, rats were deficient in vitamin E (0.8 ± 0.09 $\mu Moles.ml^{-1}$ versus 21.5 ± 0.6 $\mu Moles.ml^{-1}$ in control) when kept on vitamin E free diet for 7 months(Falanga *et al.*, 1983).

The possibility that oxygen free radicals are produced as a result of the arachidonic acid metabolism (Hertz and Cloarc, 1984; Kontos *et al.*, 1984) necessitated a study of the effects of some drugs which affect arachidonic acid metabolism on reperfusion arrhythmias. Both prostacyclin, the potent vasodilator (Bunting *et al* ., 1983) and thromboxane A_2, the powerful vasoconstrictor (Weksler, 1984) have been shown to be released from the ischaemic dog heart (Coker *et al.*, 1981; Coker and Parratt, 1983a). Furthermore, prostacyclin has been reported to be the major prostaglandin released from the isolated rat heart (De Deckere *et al.*, 1977). In anaesthetized greyhounds subjected to experimental coronary ligation and reperfusion prostacyclin has

been shown to have an antiarrhythmic effect (Coker and Parratt, 1983b) while thromboxane A_2 has been reported to exacerbate reperfusion arrhythmias (Coker *et al.*, 1982; Coker, 1984; Coker and Parratt, 1984a). The protective effect of Z.K. 36374 the more stable analogue of prostacyclin (Coker and Parratt, 1983b) against reperfusion arrhythmias in the present study is in agreement with the beneficial effect of Z.K. 36374 in anaesthetized greyhounds on reperfusion induced arrhythmias (Coker and Parratt, 1983b). In the present investigation dazoxiben, a selective inhibitor of thromboxane synthetase (Patrignani *et al.*, 1984) reduced the incidence of reperfusion arrhythmias although thromboxane A_2 has not been shown to be produced in the heart tissue (Whittle and Moncada, 1983; Weksler, 1984). On the contrary, N-butylimidazole, an imidazole derivative which also can selectively block the generation of thromboxane A_2 (Bunting *et al.*, 1983) had no effect on reperfusion induced arrhythmias in the isolated rat heart, therefore the beneficial action of dazoxiben against reperfusion arrhythmias may be due to some other mechanism other than thromboxane synthetase inhibition.

Agents like aspirin (10^{-4} M) and indomethacin (5×10^{-5} M), which inhibit the synthesis of prostaglandins (Crook *et al.*, 1976) had no effect on reperfusion induced arrhythmias in the present investigation although indomethacin showed a vasodilator effect as indicated by a marked reduction in the perfusion pressure. It seems likely that any beneficial effect of indomethacin has been masked by some other mechanism. This could be explained by what has been shown in guinea pig lung. Non-steroidal anti-

inflammatory drugs can stimulate leukotriene production by preventing the inhibitory effect of prostaglandins on leukotriene production (Kuehl *et al.*, 1984). The vasoconstrictor action of leukotriene on the coronary circulation (Piper, 1983) may argue against this possibility because in the present experiments indomethacin caused vasodilation. Indomethacin has been also reported to have no effect on ligation induced arrhythmias in anaesthetized beagles (Mullane and Moncada, 1982). In contrast, aspirin has been shown to reduce the severity of arrhythmias induced by coronary artery ligation in conscious rats (Johnston *et al.*, 1983) and anaesthetized dogs (Moschos *et al.*, 1978). These contradictory reports suggest that aspirin reduced ligation induced arrhythmias by some mechanism other than inhibition of prostaglandin synthesis.

Dexamethasone (0.2 $mg.kg^{-1}$, I.V., 1 hour before study) (Mullane *et al.*, 1984), a glucocorticoid compound which has been reported by Blackwell and Flower (1983) to interact with specific glucocorticoid receptors leading to the secretion and synthesis of protein (macrocortins) that possess antiphospholipiase properties, which will inhibit both the cyclo-oxygenase and lipoxygenase pathways due to unavailability of the substrate arachidonic acid had no effect on reperfusion induced arrhythmias. However, BW-755C a dual inhibitor of both the lipoxygenase and cyclo-oxygenase pathways of arachidonic acid metabolism (Mullane and Moncada, 1982) did reduce the severity of reperfusion arrhythmias in the present investigation. In other studies in anaesthetized dogs BW 755C has been shown to reduce ischaemic induced myocardial injury (Mullane and Moncada, 1982) and nafazatrom an antithrombotic

drug which inhibits leukotriene production also had an anti-fibrillatory effect during reperfusion of the ischaemic myocardium (Coker and Parratt, 1984b) suggesting that leukotrienes may contribute to the development of arrhythmias during myocardial infarction. The beneficial effect produced by BW 755C while dexamethasone which is also expected to inhibit both pathways of arachidonic acid had no effect suggests that BW 755C has a beneficial effect on reperfusion induced arrhythmias because its inhibitory effect on lipoxygenase pathway is predominant over the inhibition of the other pathway or that BW 755C reduced the severity of reperfusion arrhythmias by some other mechanism as scavenging of free radicals (Duniec *et al.*, 1983).

In conclusion, the present study has shown that superoxide radical is produced during reperfusion of the isolated perfused rat heart at a time when arrhythmias are initiated. This and the observation that drugs which reduce the levels of oxygen free radicals and hydrogen peroxide can protect against these arrhythmias suggest that oxygen free radicals may be important factor in the development of reperfusion induced arrhythmias *in vitro*. Agents which affect arachidonic acid metabolism yielded contradictory results suggesting that they may act by some other mechanism other than inhibition of arachidonic acid metabolism.

CHAPTER 6

"Effect of some vasodilators on reperfusion induced arrhythmias in the isolated rat heart"

Section A: Results

In the previous two chapters of the present study potassium, magnesium and glutathione reduced the perfusion pressure in the isolated rat heart. This vasodilation might be expected to have affected the development of ischaemically induced damage following coronary artery ligation and this in turn could have indirectly affected the severity of reperfusion arrhythmias. Therefore it was of interest to try to study the effect of some vasodilators on reperfusion induced arrhythmias.

From Figs. (67) - (69) it can be seen that all the vasodilators used, adenosine (10^{-6} M, which was found to produce the maximal vasodilation), sodium nitroprusside (10^{-6} M) and Z.K. 36374 (10^{-9} M) reduced the incidence of VF while verapamil (10^{-7} M) completely prevented the development of VF and reduced the incidence of VT. There was no other significant effect on the incidence of VT, the onset and duration of VT and VF or the number of PVCs. As in Fig. (70) the vasodilators in the concentrations used reduced the perfusion pressure but had no effect on developed tension or heart rate except verapamil (Chapter 4.11) which reduced the perfusion pressure, developed tension and heart rate.

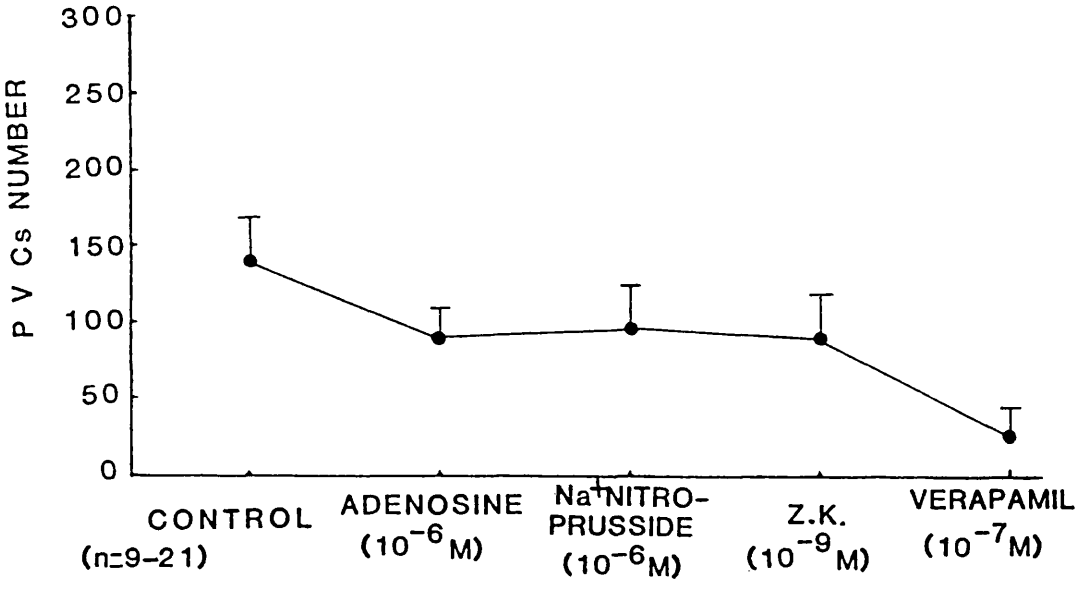


Fig. (67). Effect of adenosine (10^{-6}M), sodium nitroprusside (10^{-6} M), Z.K. 36374 (10^{-9} M) and verapamil (10^{-7} M) on the number of PVCs developed during reperfusion in the isolated rat heart. Potassium 3.2 mM magnesium 1.2 mM and calcium 1.2 mM.

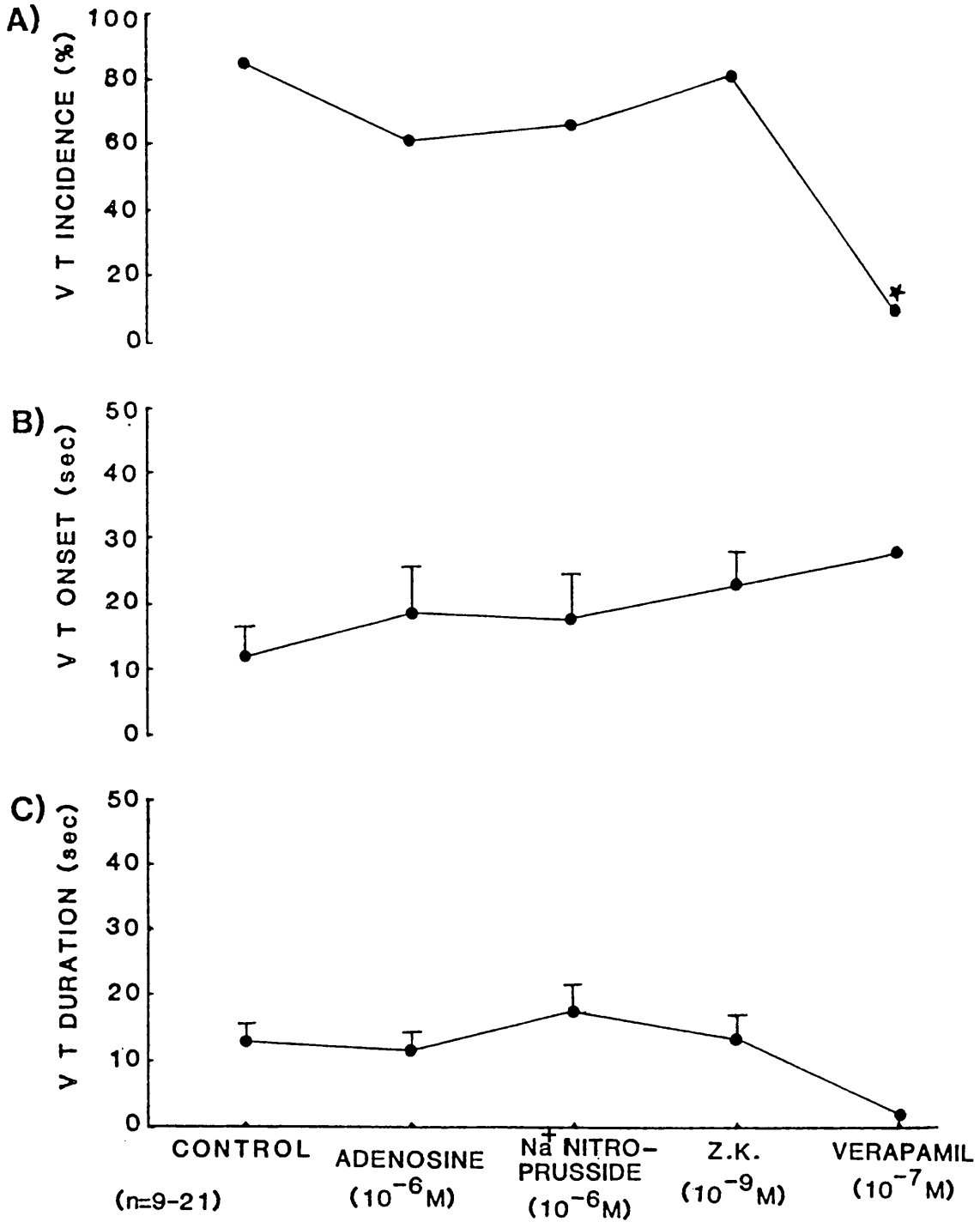


Fig. (68). Effects of adenosine (10^{-6} M), sodium nitroprusside (10^{-6} M), Z.K. 36374 (10^{-9} M) and verapamil (10^{-7} M) on a) the incidence, (b) onset and (c) duration of VT developed during reperfusion. Potassium 3.2 mM .

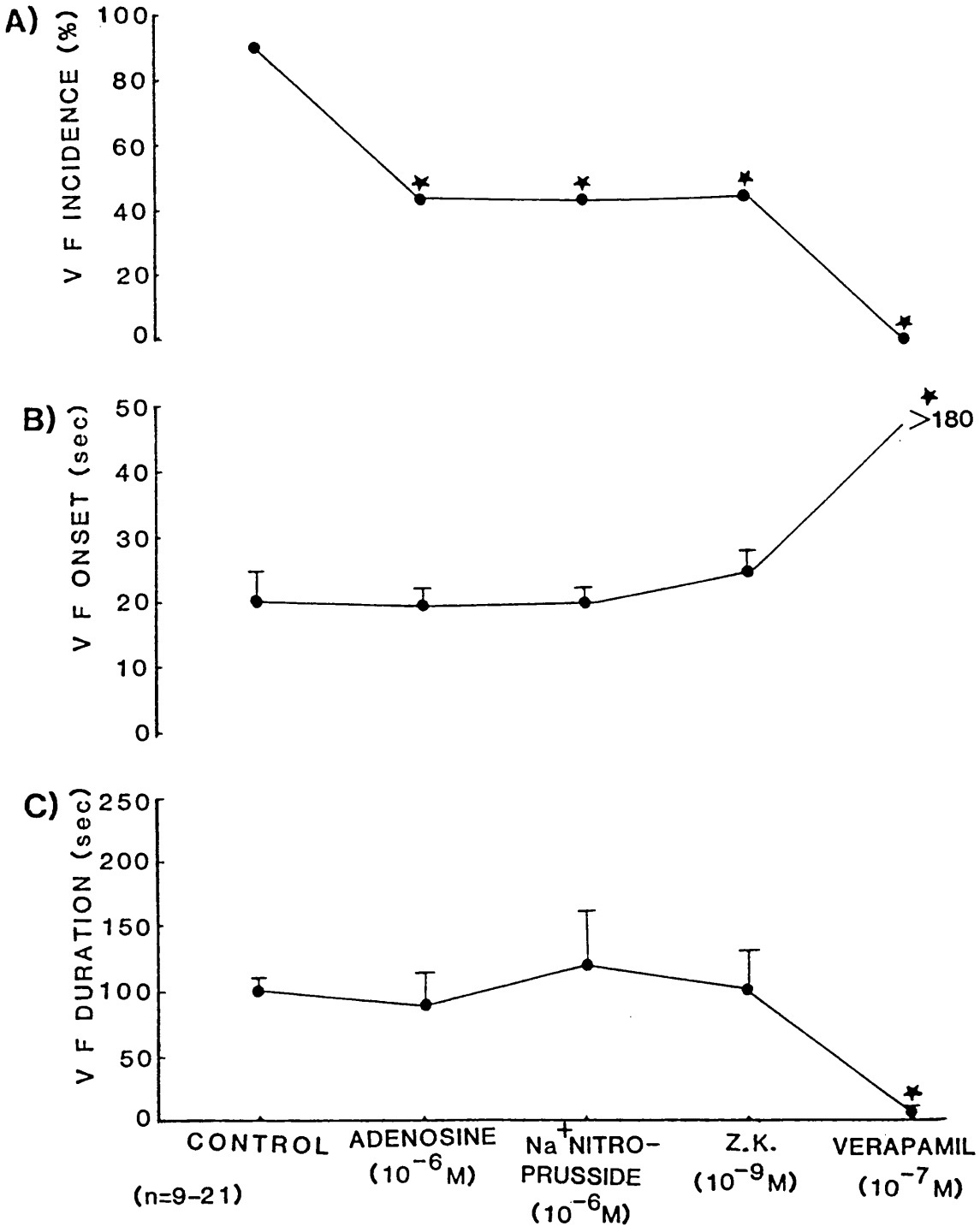


Fig. (69). Effects of adenosine (10^{-6} M), sodium nitroprusside (10^{-6}M) Z.K. 36374 (10^{-9}M) and verapamil (10^{-7}M) on a) the incidence, b) onset and c) duration of VF developed during reperfusion. Potassium 3.2 mM.

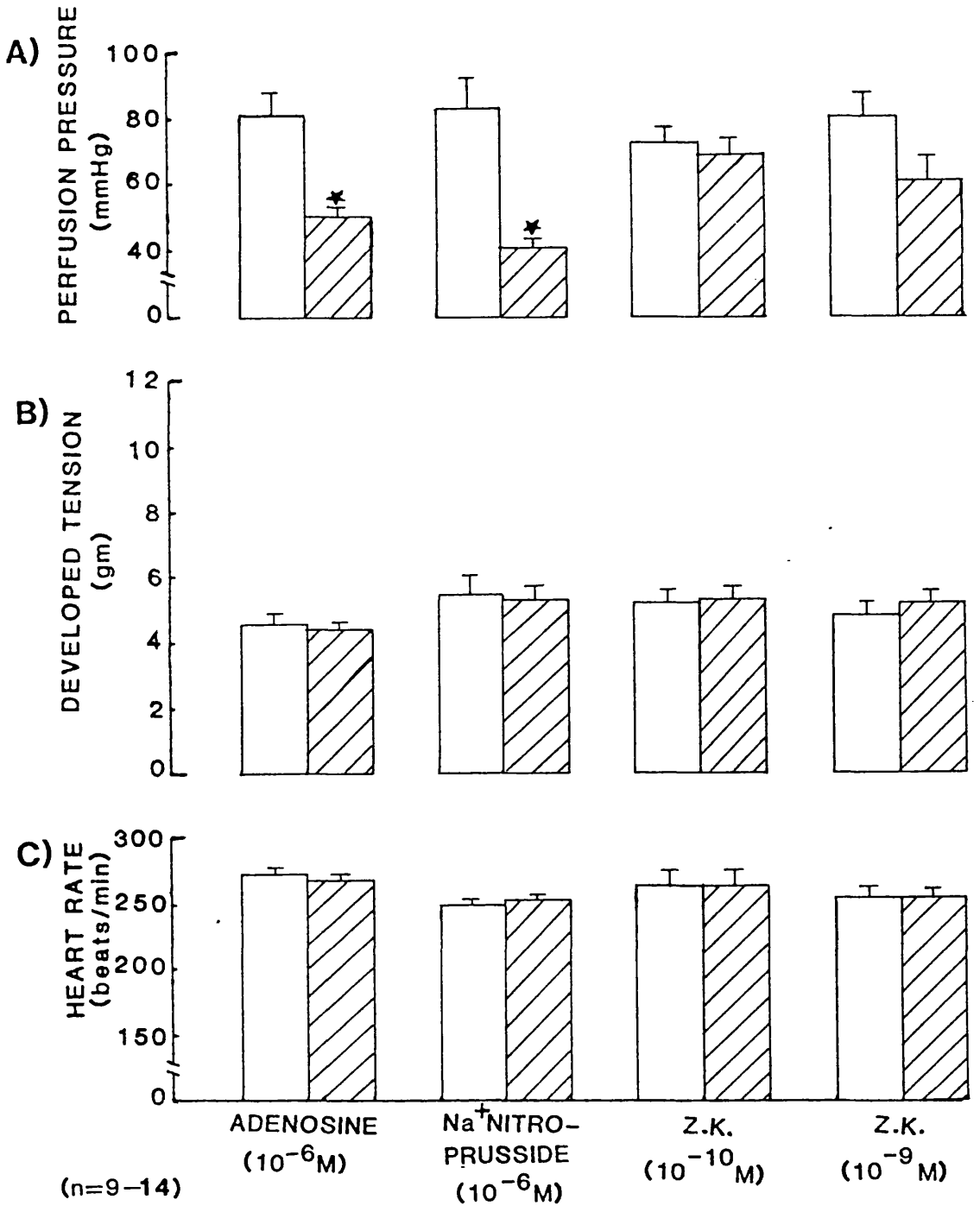


Fig. (70). Effects of adenosine (10^{-6} M), sodium nitroprusside (10^{-6}M), Z.K. 36374 (10^{-10}M and 10^{-9}M) on (a) perfusion pressure, (b) developed tension and(c) heart rate in the isolated rat heart. Potassium 3.2 mM; magnesium 1.2 mM and calcium 1.2 mM.

Section B: Discussion

Vasodilation occurring during the pre-ligation period is expected to affect the development of ischaemically induced damage following coronary artery ligation and this in turn may affect the severity of arrhythmias developing on reperfusion. In addition, vasodilation could influence the distribution of coronary flow and rate of reperfusion of the ischaemic tissue via a coronary steal effect. These two possibilities suggested the confirmation of the effect of some vasodilators on reperfusion induced arrhythmias.

In the present study, the significant reduction in the incidence of reperfusion induced VF produced by adenosine, sodium nitroprusside, Z.K. 36374 and verapamil supports the previous concept that coronary vasodilation may protect against reperfusion induced arrhythmias by reducing the ischaemic damage due to an anti-ischaemic effect. However, as a constant flow system was used in these experiments and the fact that there is very little collateral circulation in the rat heart suggest that this is unlikely as total oxygen delivery to the heart is unchanged. It is possible that the beneficial action of vasodilators is due to an effect on the rate of reperfusion. Dilating coronary vessels in the constant flow perfusion system reduces perfusion pressure by withdrawal of the perfusate to the non-ischaemic area and this will in turn reduce the speed at which reperfusion takes place. Consistent with the latter concept are the findings that in the dog slow reperfusion of ischaemic myocardium reduces the severity of reperfusion induced arrhythmias (Sewell *et al.*,

1955). Furthermore, in the present study when reperfusion was induced at a slow rate (Chapter 4: Figs. (33) - (35)) the incidence of the resulting arrhythmias was markedly reduced. On the other hand, in a recent study in the anaesthetized rat the incidence of reperfusion induced arrhythmias was unaffected by the administration of nitroglycerine, the coronary dilating agent (Kane *et al.*, 1984). This ineffectiveness of nitroglycerine in the *in vivo* model may be due to the presence of extracardiac neural and hormonal reflex mechanisms and also because coronary flow can vary in this model.

In conclusion, in the present study, in the isolated rat heart using a constant flow system vasodilators markedly reduced the incidence of reperfusion arrhythmias. It seems likely that this effect is being produced via a coronary steal effect.

CHAPTER 7

"Effects of oxygen free radicals on potassium efflux and noradrenaline release on reperfusion after 10 min ischaemia in the isolated rat heart"

Section A: Results

Oxygen free radicals generated during myocardial ischaemia and reperfusion are expected to induce peroxidation of cell membrane lipids leading to a reduction in its function and increased efflux of intracellular potassium and influx of extracellular sodium. Washout of potassium on reperfusion has been reported to cause electrophysiological derangements and probably contributes to the development of reperfusion induced arrhythmias (see Chapter 1.3). Therefore, it was important to study the effect of free radical generation as well as free radical scavengers on the efflux of potassium in order to find out if there is a reduction in potassium efflux produced by free radical scavengers and if there is a relation between this effect and the antiarrhythmic action of the free radical scavengers mentioned in Chapter 5. In these experiments, ^{86}rubidium was used because it behaves as potassium and it has a longer half life compared with the very short half life of ^{42}potassium which makes its use inconvenient.

7.1: Effect of free radical generation on ^{86}rubidium efflux

From Fig. (71b) it can be seen that generation of superoxide radical by xanthine/xanthine oxidase system produced non significant transient increase in $^{86}Rb^{+}$ efflux (Fig. (71b)). This increase was reduced in the presence of the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) while this increase was completely prevented when the enzyme xanthine

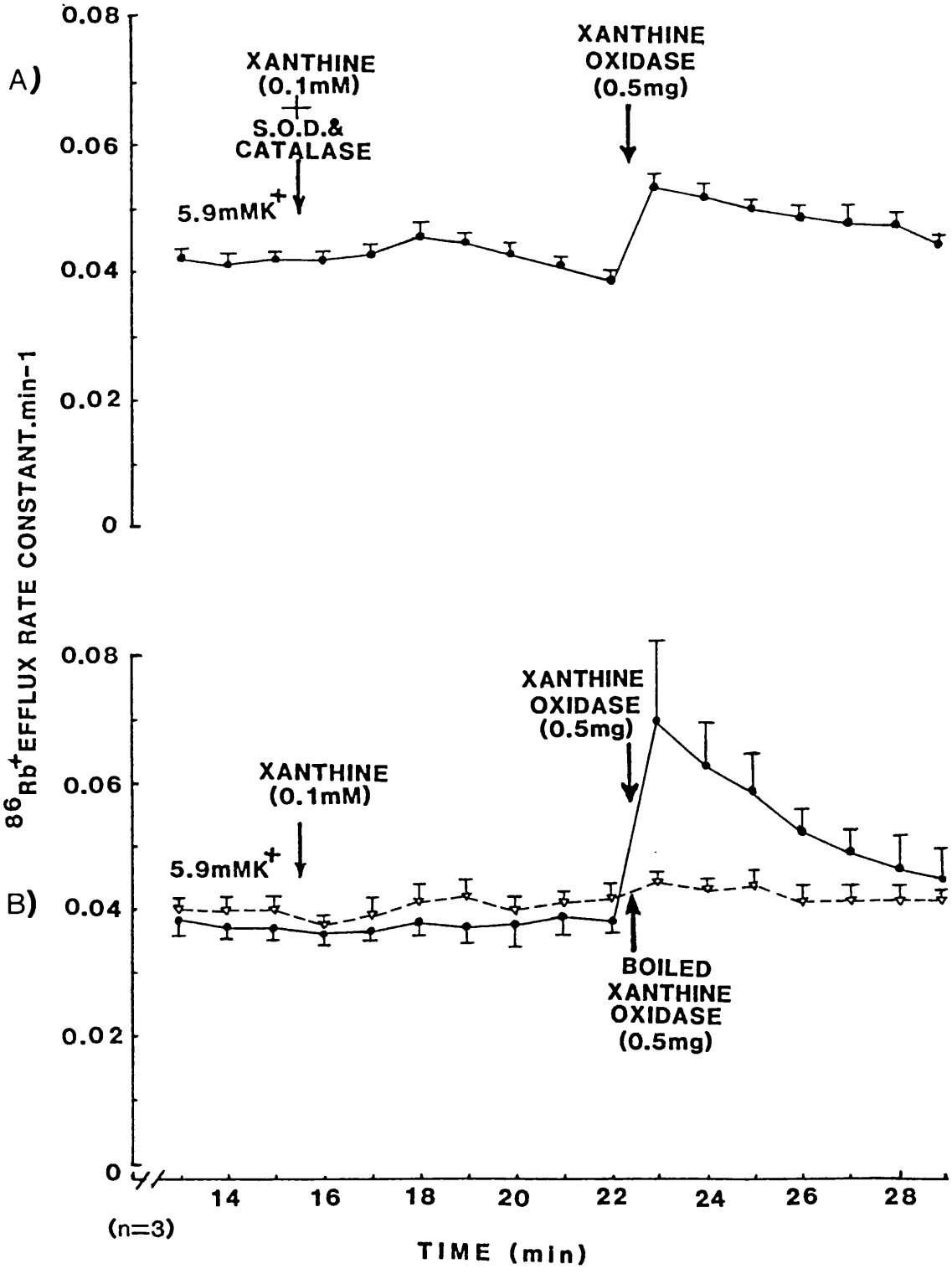


Fig. (71). Effect of xanthine (10^{-4}M)/xanthine oxidase (single injection of 0.5 mg) on $^{86}Rb^{+}$ efflux rate constant in the presence of: (a) a mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) (b) in absence of free radical scavengers using active (●—●) and heat inactivated (▽--▽) enzyme in the isolated rat heart.

oxidase was boiled for 5 minutes (Fig. (71b)). Hydrogen peroxide (3×10^{-5}, 6×10^{-5} and 12×10^{-5} M) had no significant effect on $^{86}Rb^{+}$ efflux (Fig. (72a)). Similar to hydrogen peroxide, neither ferrous ion nor the mixture of ferrous ion plus hydrogen peroxide had any significant effect on $^{86}Rb^{+}$ efflux in the isolate rat heart (Fig. (72b)).

7.2: Efflux rate constant of $^{86}Rb^{+}$ during the course of 10 min ischaemia and 3 min reperfusion in the isolated rat heart

As in Fig. (73) when the potassium concentration used was 10 mM, there was a non significant transient increase in $^{86}Rb^{+}$ efflux on reperfusion after 10 min ischaemia. When the concentration of potassium was reduced to 3.2 mM in order to increase the myocardial cell damage on coronary artery ligation and reperfusion, there was a significant increase in $^{86}Rb^{+}$ efflux rate constant on reperfusion which was sustained over the three minutes of reperfusion (Fig. (74)). This increase in $^{86}Rb^{+}$ efflux rate constant is expected to be due to the process of reperfusion itself because if it is due to the washout of $^{86}Rb^{+}$ released during the ischaemic period it would have come down after 1 min as in the case of the washout of lactate which increased in the reperfusate during the first minute only (Chapter 5: Table 14).

7.3: Effect of glutathione on $^{86}Rb^{+}$ efflux rate constant during coronary artery ligation and reperfusion

In Fig. (75b) it can be seen that glutathione (10^{-5}, 10^{-4} and 10^{-3} M) produced a concentration dependent reduction in the

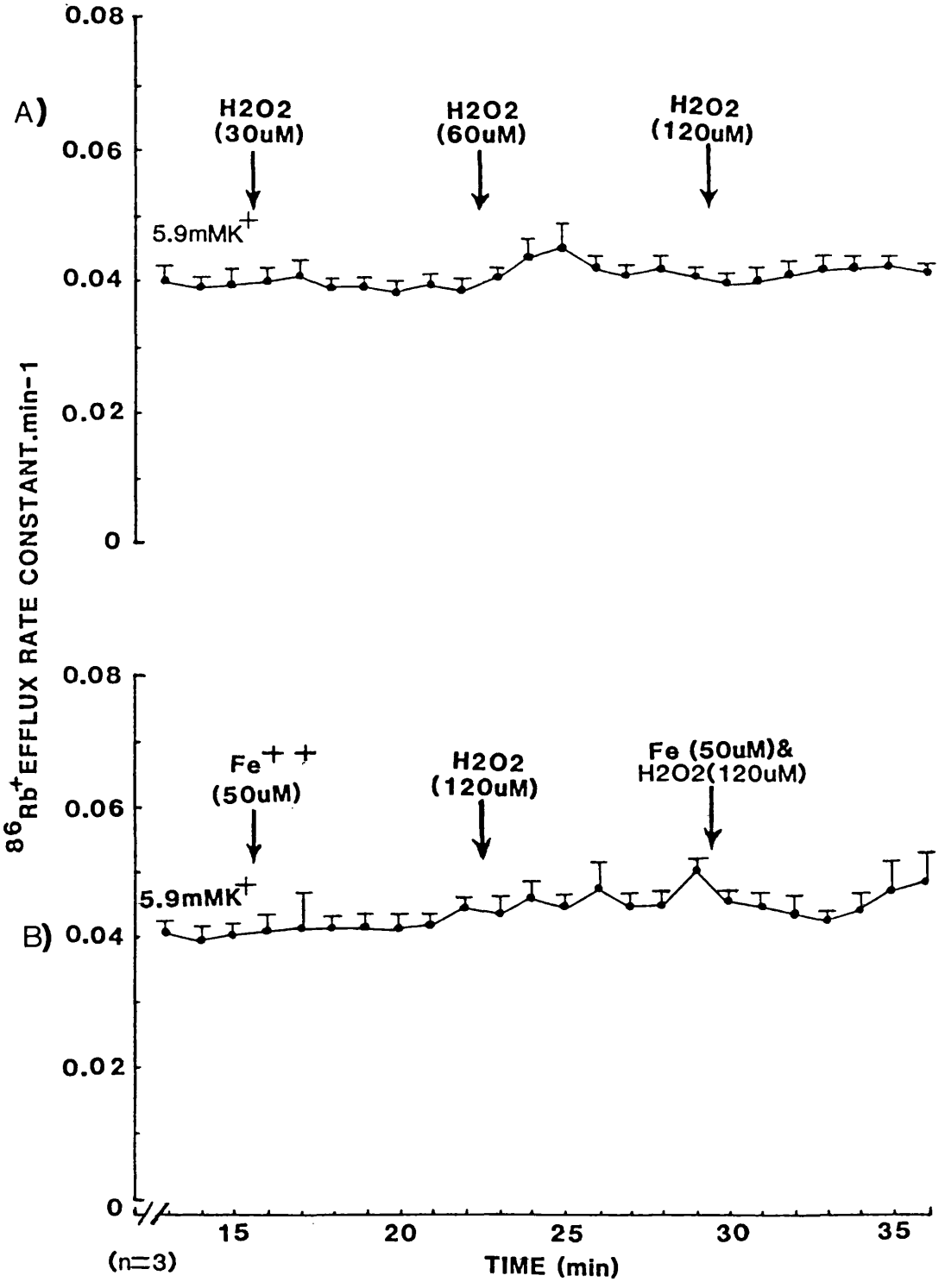


Fig. (72). The efflux rate constant of $^{86}Rb^{+}$ in the isolated rat heart in the presence of: (a) $3x10^{-5}$M H_2O_2 for 7 min, then $6x10^{-5}$M H_2O_2 for 7 min, then $12x10^{-5}$M H_2O_2 for 7 min. (b) $5x10^{-5}$M ferrous ion for 7 min, $12x10^{-5}$M H_2O_2 for 7 min and the mixture of ferrous ion and H_2O_2 for 7 minutes.

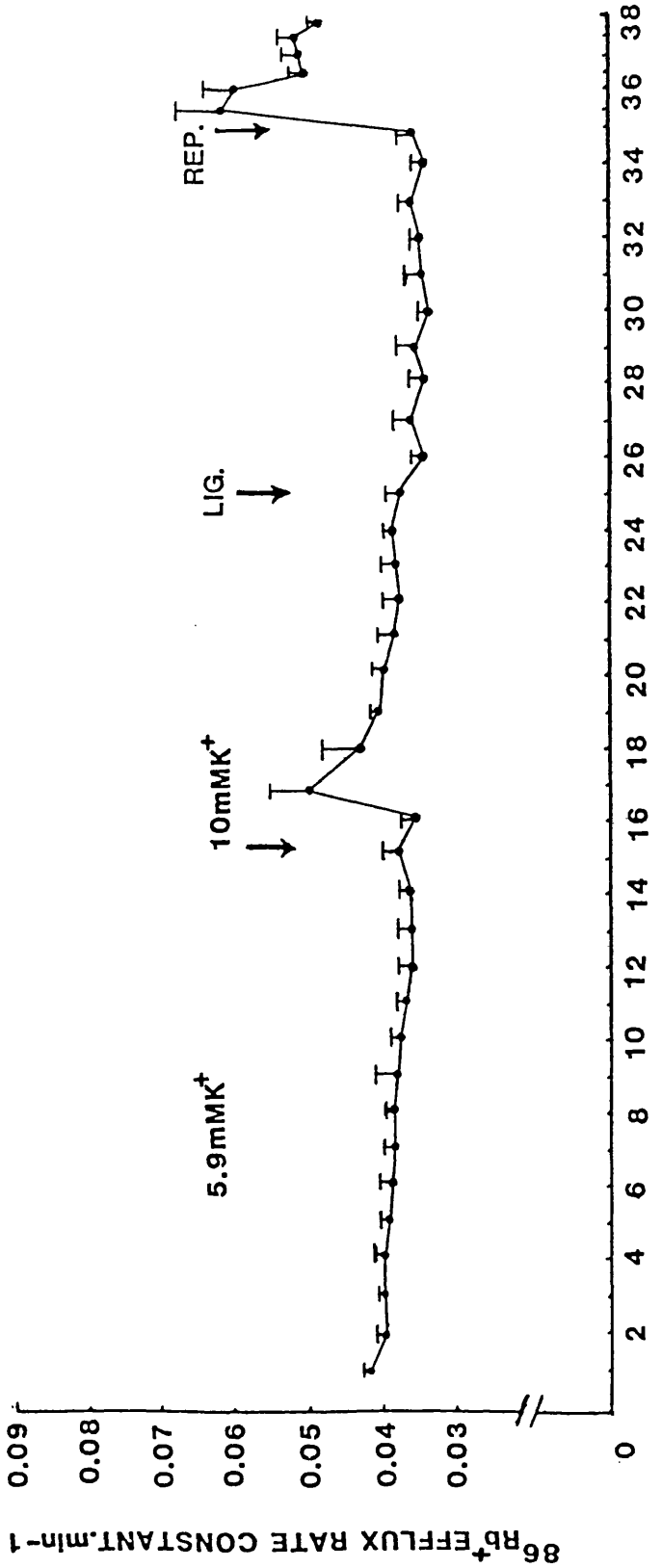


Fig. (73). The time course of the change in $^{86}Rb^{+}$ efflux rate constant.min^{-1} during 10 min ischaemia and reperfusion in the isolated rat heart. The concentration of potassium was increased from 5.9 mM to 10 mM at the time shown by the arrow.

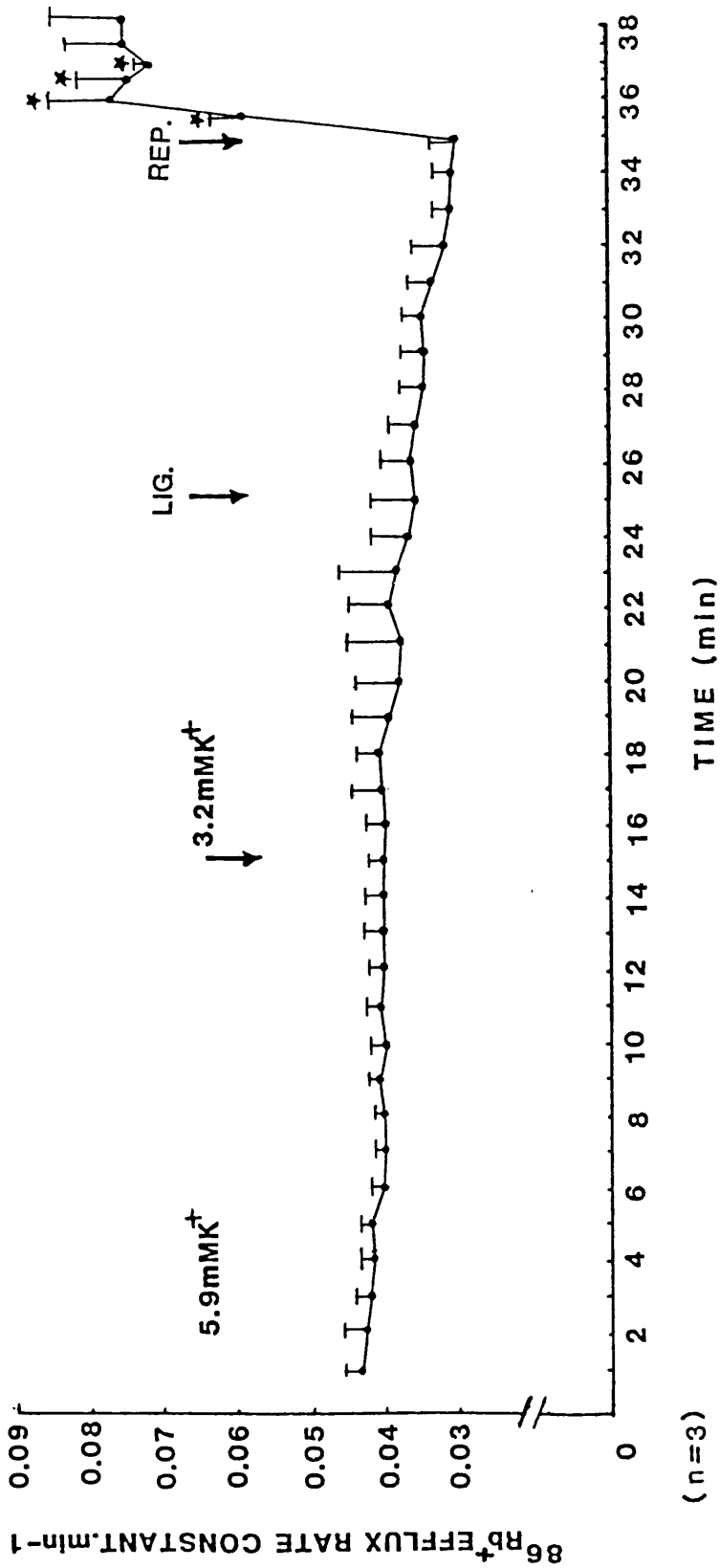


Fig. (74). The time course of the change in $^{86}Rb^{+}$ efflux rate constant.min^{-1} during 10 min ischaemia and reperfusion in the isolated rat heart. The concentration of potassium was reduced from 5.9 mM to 3.2 mM at the time shown by the arrow. * $P < 0.05$.

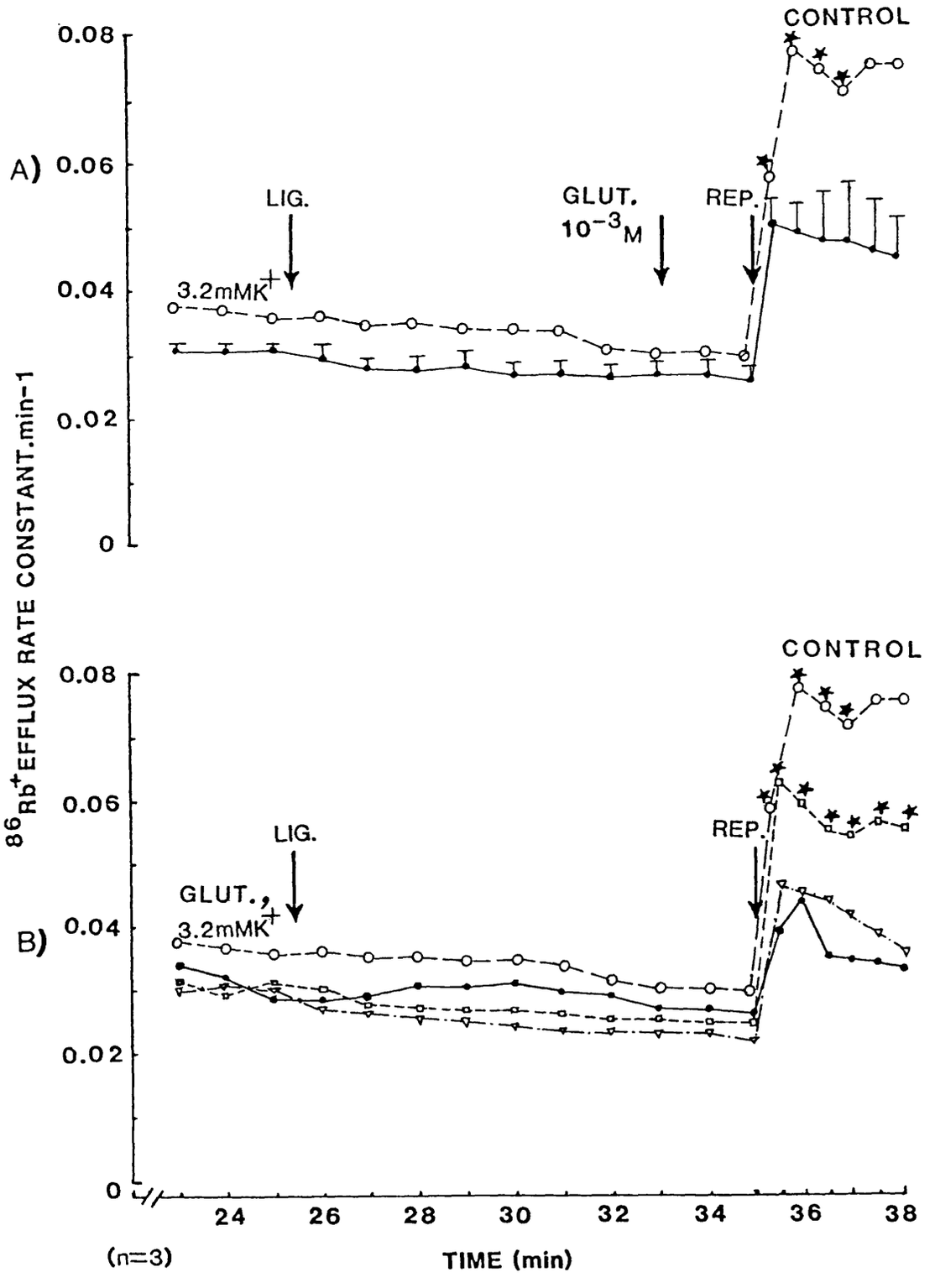


Fig. (75). Effect of glutathione (10^{-5}, 10^{-4} and 10^{-3}M) on the $^{86}Rb^{+}$ efflux rate constant during 10 min coronary ligation and 3 min reperfusion when given (a) 8 min after coronary ligation, (b) 5 min before coronary ligation (10^{-5}M: ▫---▫ 10^{-4}M; ▽---▽ and 10^{-3} M:●—●) in the isolated rat heart. *P $<$ 0.05 In (b) S.E.M.s were excluded for clarity.

efflux rate constant of $^{86}Rb^{+}$ on reperfusion. The increase of $^{86}Rb^{+}$ produced on reperfusion in the control group was reduced to about one third in the presence of 10^{-3} and 10^{-4} M glutathione and it became non significantly different from the pre-ligation values. In the presence of 10^{-5} M glutathione the increase in $^{86}Rb^{+}$ efflux rate constant on reperfusion was still significantly different from the pre-ligation values although it was reduced to about two thirds of the control values. When glutathione (10^{-3} M) was given 8 min after coronary ligation, it still reduced the increase in $^{86}Rb^{+}$ efflux rate constant produced in control group during reperfusion to about 50% of its value which became non significantly different from the pre-ligation values (Fig. (75a)). Glutathione (10^{-3} M) has a potent vasodilator action. This could affect the rate of efflux of $^{86}Rb^{+}$ during myocardial ischaemia due to the reduction of the ischaemic damage by increasing the collateral flow to the ischaemic area. Also, this vasodilator effect could reduce the rate of washout of released $^{86}Rb^{+}$ during the ischaemic period by reduction of the rate of reperfusion via the coronary steal effect. Therefore, in order to find out if the effect of 10^{-3} M glutathione is produced in part by its vasodilator effect, the effects of another vasodilator adenosine (10^{-6} M) on $^{86}Rb^{+}$ efflux rate constant were examined during myocardial ischaemia and reperfusion (Fig. (76)). From Fig (76) it can be seen that adenosine (10^{-6} M) which produces maximal vasodilation had a similar effect to that produced by glutathione (10^{-3} M) when used either 5 min before ligation or 2 min before reperfusion. From Figs. (75) and (76) it can be seen that little difference between the effects of glutathione (10^{-3} M) and adenosine

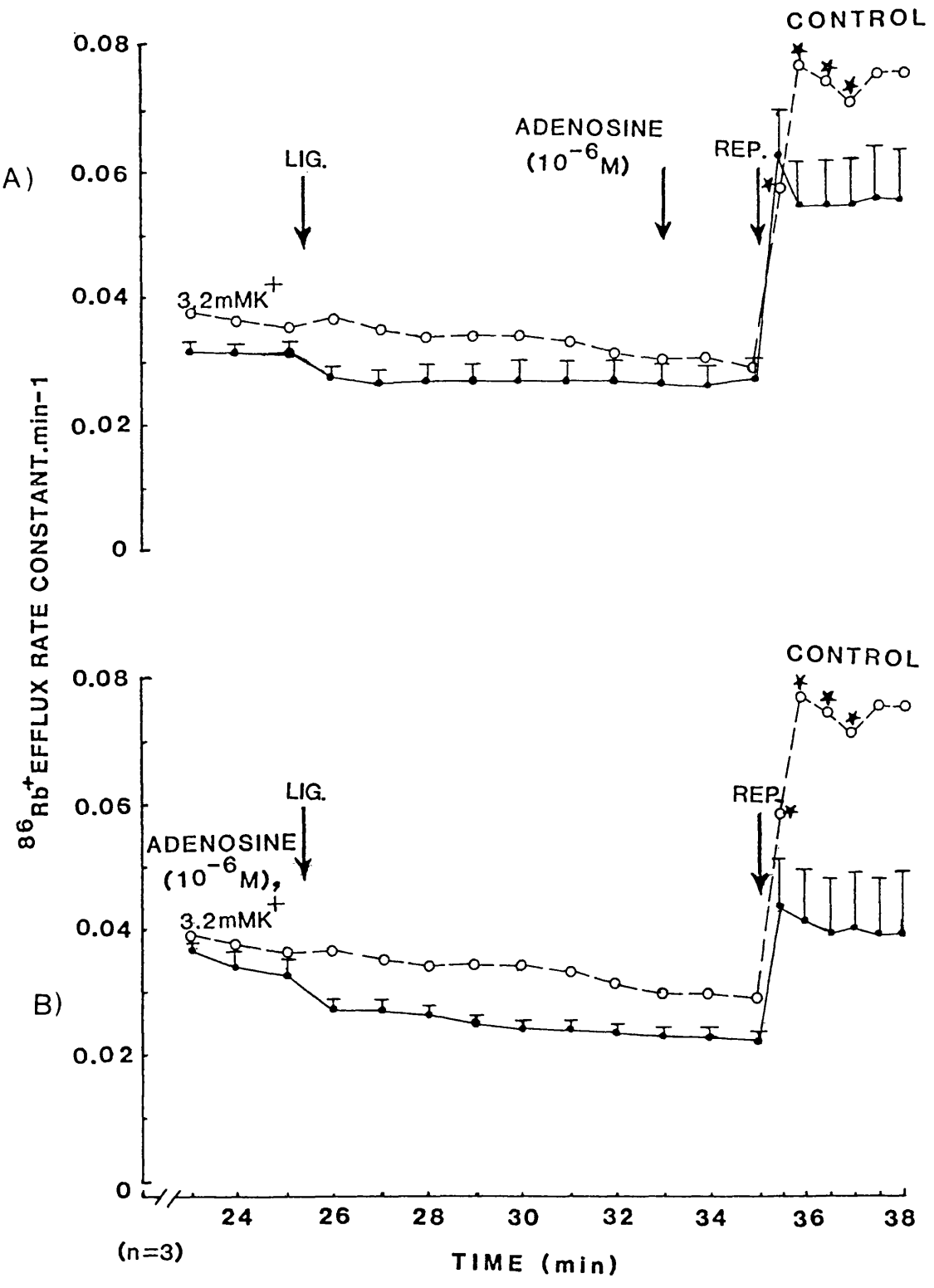


Fig. (76). Effect of adenosine (10^{-6} M) on $^{86}Rb^{+}$ efflux rate constant during 10 min coronary ligation and 3 min reperfusion when given: (a) 8 min after coronary ligation, (b) 5 min before coronary ligation in the isolated rat heart. *P < 0.05.

(10^{-6} M) on $^{86}Rb^{+}$ efflux rate constant when given before coronary ligation or just before reperfusion.

7.4: Effect of the mixture of superoxide dismutase plus catalase plus mannitol on $^{86}Rb^{+}$ efflux rate constant during coronary artery ligation and reperfusion

In the presence of the mixture of superoxide dismutase plus catalase plus mannitol, there was a transient increase in $^{86}Rb^{+}$ efflux rate constant during the first minute of reperfusion however, this was not sustained during the last two minutes of reperfusion (Fig. (77)). This transient increase seems more likely to be due to washout of the $^{86}Rb^{+}$ released during ischaemic period.

7.5: Effect of the mixture of superoxide dismutase plus catalase plus mannitol on the time course of ^{3}H-noradrenaline release rate constant during 10 min coronary ligation and 3 min reperfusion in the isolated rat heart

From Fig. (78) it can be seen that there was a transient non significant increase in ^{3}H-noradrenaline concentration in the perfusate during the first minute of reperfusion. This could be due to increased release of noradrenaline on reperfusion or washout of noradrenaline released during the ischaemic period. When the mixture of superoxide dismutase plus catalase plus mannitol was added to the perfusate 5 min before coronary artery ligation, this increase in noradrenaline concentration in the perfusate on reperfusion was prevented. 10 mM potassium

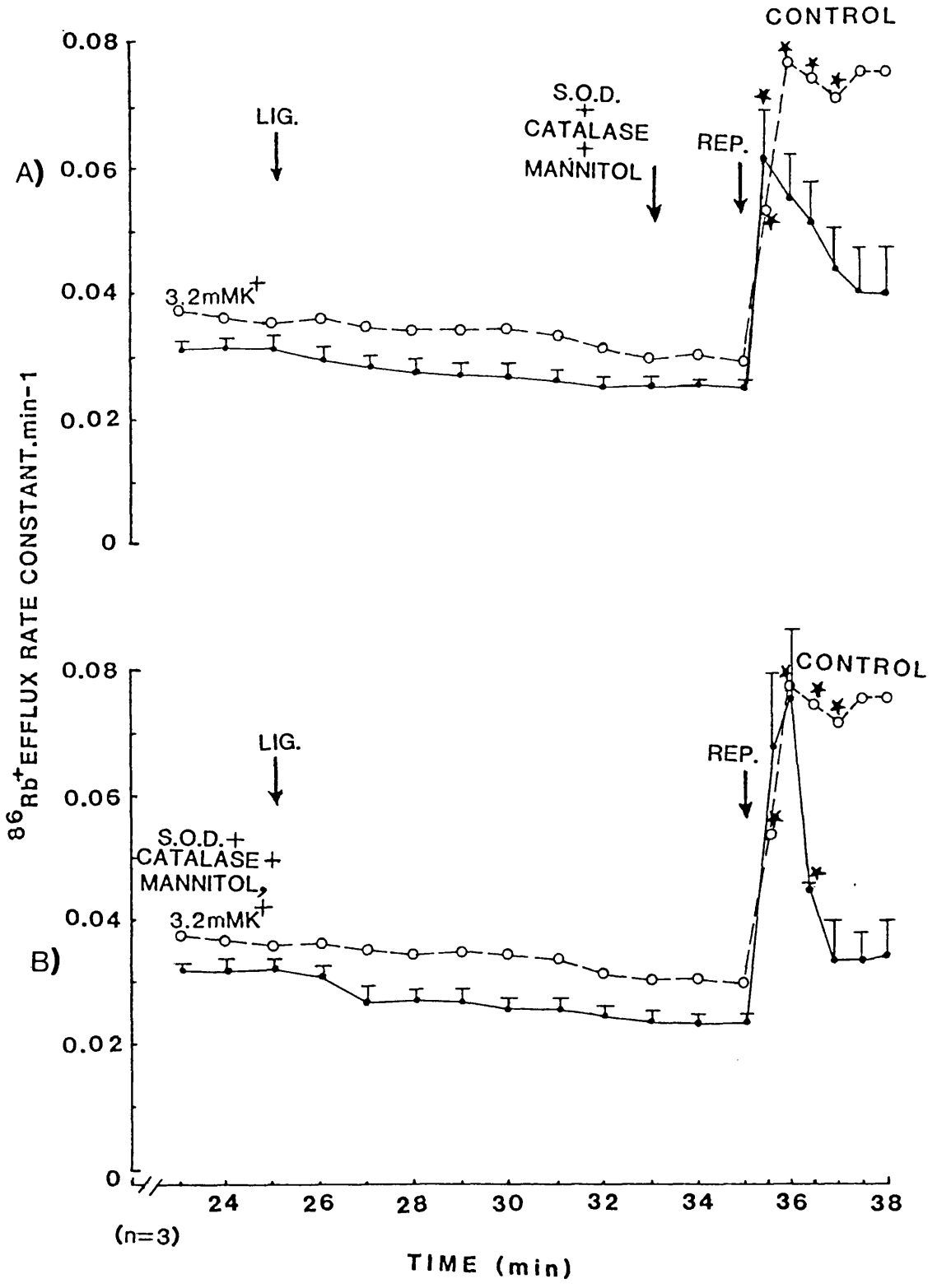


Fig. (77). Effect of the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) plus mannitol ($2x10^{-2}$M) on the time course of $^{86}Rb^{+}$ efflux rate constant during 10 min coronary ligation and 3 min reperfusion when given: (a) 8 min after coronary ligation, (b) 5 min before coronary ligation in the isolated rat heart. *P < 0.05.

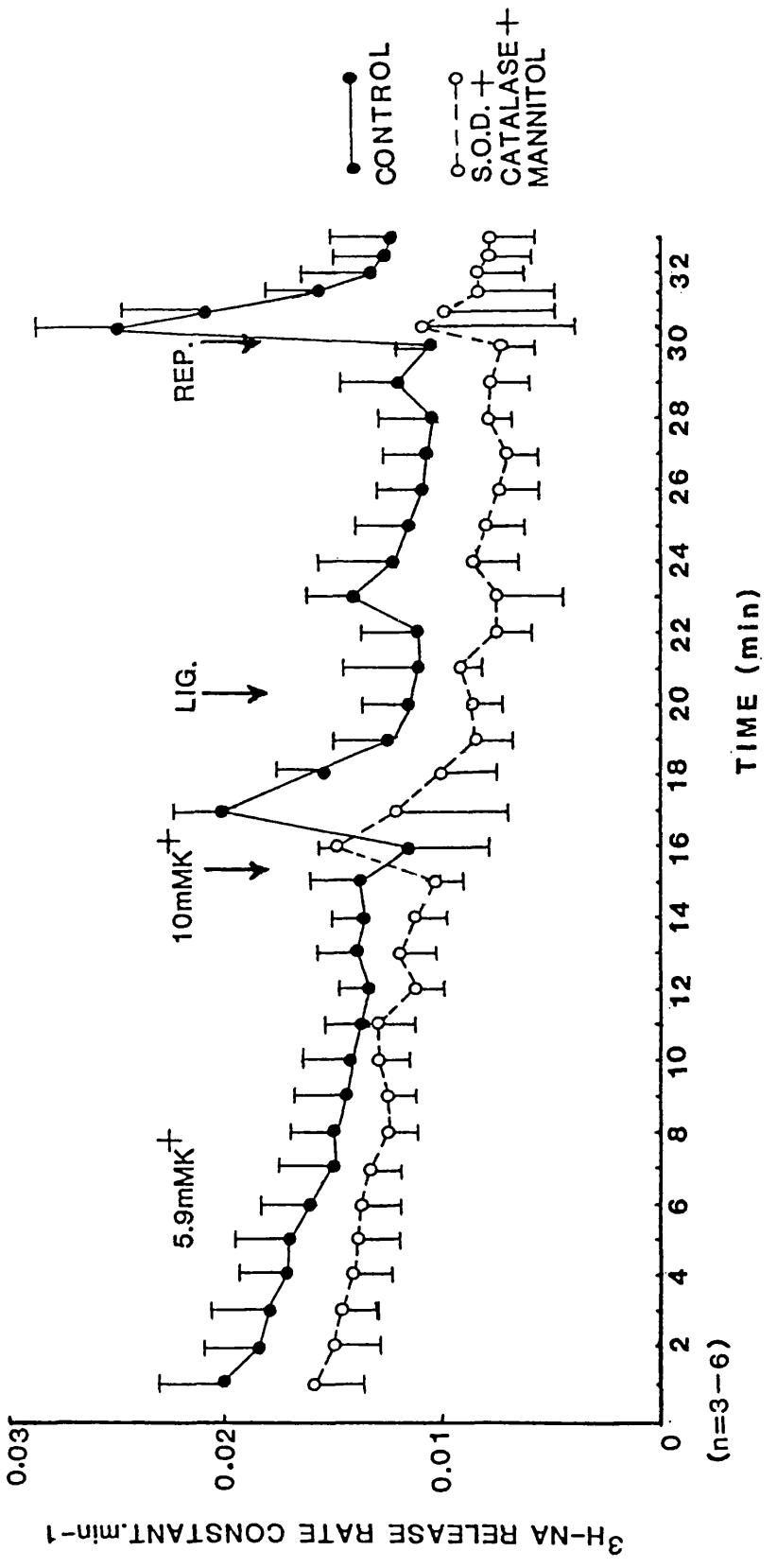


Fig. (78). Effect of the mixture of superoxide dismutase (10 units.ml^{-1}) plus catalase (100 units.ml^{-1}) plus mannitol (2 x 10^{-2} M) on the time course of ^{3}H-noradrenaline release rate constant.min^{-1} during 10 min coronary ligation and 3 min reperfusion in the isolated rat heart.

concentration was used in order to prevent the incidence of VF.

Section B: Discussion

Oxygen free radicals are expected to affect the efflux of intracellular potassium during myocardial ischaemia and reperfusion due to reduced function and integrity of the sarcolemma induced by peroxidation of cell membrane lipids (Meerson *et al.*, 1982). The wash out of the accumulated potassium on reperfusion has been reported to induce electrophysiological derangements and contribute to the development of reperfusion arrhythmias (Corr and Witkowski, 1984). In order to investigate the effect of free radical scavengers on potassium efflux during myocardial ischaemia and reperfusion it was inconvenient to use ^{42}potassium because of its very short half-life time (12.4 hours). Although rubidium had been reported to interfere with the inward movement of potassium ions (Adrian, 1964) radioactive rubidium ($^{86}Rb^{+}$) has been used as a tracer of potassium ions for investigations in secretory tissues of rat and mouse pancreatic islets (Henquin, 1977; Matthews, and Shotton, 1984) and tissues of membrane transport systems such as the sodium-potassium pump (Tomita and Tamamoto, 1971; Imaizumi and Watanabe, 1981). Therefore, ^{86}rubidium has been used to trace the efflux of potassium during 10 min ischaemia and reperfusion in the isolated rat heart in the present study. Superoxide radical generation by xanthine (10^{-4} M)/xanthine oxidase (0.5 mg) (Hess *et al.*, 1981) produced a sudden increase in ^{86}rubidium efflux rate which returned back to the control

levels after seven minutes. This effect was reduced by about 50% in the presence of the mixture of superoxide dismutase and catalase and was completely prevented by deactivation of the enzyme xanthine oxidase by boiling suggesting that this effect on ^{86}rubidium efflux is due to the enzymatic activity of xanthine oxidase and superoxide anion production. This increase in rubidium efflux in the presence of the mixture of superoxide dismutase and catalase may be due to excess superoxide anion production suggesting that the concentrations of superoxide dismutase and catalase were insufficient or they do not get into cells. The effect of xanthine/xanthine oxidase system on ^{86}rubidium efflux provides evidence that superoxide generation can induce cell membrane damage and reduced membrane function. However, hydrogen peroxide and the mixture of hydrogen peroxide plus ferrous ion did not increase ^{86}rubidium efflux suggesting that the presence of superoxide anion in the reaction medium is necessary to induce damage. This may also negate what has been reported by Halliwell and Gutteridge (1984) that a simple mixture of hydrogen peroxide and ferrous salt can produce hydroxyl radicals via Fenton's reaction.

When the effects of myocardial ischaemia and reperfusion were compared in two potassium concentrations (10 mM and 3.2 mM) a higher increase in $^{86}Rb^{+}$ efflux was produced when 3.2 mM potassium was being used. This low potassium concentration also exacerbates arrhythmias during reperfusion. This increased

$^{86}Rb^{+}$ efflux with a low potassium concentration could be due to the more severe arrhythmias which develop with low concentrations of potassium (Chapter 4). High extracellular potassium concentrations have been reported to increase potassium efflux in the smooth muscle preparation (Bolton and Clarke, 1981).

The beneficial antiarrhythmic effects of glutathione and the mixture of superoxide dismutase plus catalase plus mannitol were associated with a reduced efflux of $^{86}Rb^{+}$ during reperfusion, this provides further evidence that oxygen free radicals might be involved in inducing cell membrane damage on reperfusion of ischaemic myocardium. Because the highest concentration of glutathione (10^{-3} M) has a potent vasodilator action which in turn can reduce the rate of reperfusion leading to a slower washout of accumulated $^{86}Rb^{+}$, the vasodilator adenosine was also given before coronary ligation and 2 min before reperfusion. It can be seen from the present study that adenosine had similar but slightly weaker effect on $^{86}Rb^{+}$ efflux rate when compared with that produced by glutathione. Therefore, the vasodilator action of glutathione is probably contributing to its effect on $^{86}Rb^{+}$ efflux rate constant. The increase in $^{86}Rb^{+}$ efflux rate constant in the presence of the mixture of superoxide dismutase plus catalase plus mannitol produced on reperfusion was transient as in the case of the washout of lactate which increased in the reperfusate during the first minute only (Table 14) suggesting that the increase in $^{86}Rb^{+}$ efflux rate seen in the presence of the mixture of superoxide dismutase plus catalase plus mannitol is due to a sudden washout of $^{86}Rb^{+}$ accumulated during myocardial ischaemia due to the depletion of high energy nucleotide pools

(Downar *et al.*, 1977a; Swain *et al.*, 1984) while the sustained and reduced increase in $^{86}Rb^{+}$ efflux during the first three minutes of reperfusion in the presence of glutathione (10^{-3} M) or adenosine may be due to that accumulated $^{86}Rb^{+}$ during the ischaemic period has been washed out at a slower rate over the three minutes period of reperfusion due to a coronary steal effect.

The mixture of superoxide dismutase plus catalase plus mannitol also reduced the transient increase in ^{3}H-noradrenaline release during reperfusion of ischaemic myocardium suggesting another mechanism by which free radical scavengers might produce their beneficial action. It is possible that free radicals damage nerve terminals to increase catecholamine release and these could then contribute to arrhythmias.

In conclusion, the increase in $^{86}Rb^{+}$ efflux rate on reperfusion could be due to washout of accumulated $^{86}Rb^{+}$ due to Na^{+}-K^{+} pump inhibition during myocardial ischaemia and/or passive efflux caused as a result of reduced membrane integrity due to free radical induced membrane damage. The present study revealed that oxygen free radical scavengers can protect against the second mechanism as well as preserve the nerve endings from the damaging effect of free radicals.

CHAPTER 8

"Conclusion and suggestions for future work"

8.1: Conclusion

The present study demonstrates that reperfusion induced cardiac arrhythmias can be reliably and consistently produced in the perfused rat heart under constant flow conditions . The severity of these arrhythmias is maximum when reperfusion occurs after 10 minutes of myocardial ischaemia and they are not significantly different from those produced in a constant pressure system or in anaesthetiSed rat models.

Reperfusion arrhythmias in the isolated rat heart model are significantly affected by the ionic environment of the heart (reperfusion arrhythmias increase by increasing the extracellular concentration of potassium and/or magnesium and decrease by increasing the extracellular concentration of calcium). If a transient hypokalaemia occurred in the presence of mild hypomagnesaemia or hypercalcaemia then these changes could be an important factor in determining the severity of any reperfusion arrhythmias that might occur in man.

The present results suggest that perfusion pressure, developed tension, heart rate and the rate of reperfusion can affect the severity of reperfusion induced arrhythmias. Agents which reduced perfusion pressure, developed tension or heart rate attenuated the severity of reperfusion induced arrhythmias except the case of indomethacin which reduced perfusion pressure but had no effect on the incidence of arrhythmias. This could be due to the fact that indomethacin by its inhibitory effect on cyclooxygenase enzyme would stimulate leukotriene production through the lipoxygenase pathway and this could have masked any

beneficial effect of vasodilation produced by indomethacin. The protective effect of vasodilators against reperfusion arrhythmias seems to be produced by decreasing the rate of reperfusion via a coronary steal effect. Some of the agents which affect arachidonic acid metabolism have beneficial effects on reperfusion arrhythmias which may be due to mechanisms other than inhibition of arachidonic acid metabolism.

Superoxide radicals are being produced during reperfusion of the isolated rat heart at the time when arrhythmias are initiated. Drugs which reduce the levels of oxygen free radicals and hydrogen peroxide can protect against reperfusion induced arrhythmias suggesting that oxygen free radicals may be an important factor in the development of these arrhythmias in the isolated rat heart.

Oxygen free radicals produced by xanthine/xanthine oxidase system or on reperfusion of ischaemic myocardium can induce cell membrane damage and reduced membrane function as indicated by increased $^{86}Rb^{+}$ efflux rate constant and this effect can be prevented by free radical scavengers. The present data provides evidence for the involvement of oxygen free radicals in the induction of cell membrane damage which could somehow be involved in the development of reperfusion induced arrhythmias.

8.2: Suggestions for future work

- To investigate the effects of agents which affect arachidonic acid metabolism in more detail and in different doses on reperfusion induced arrhythmias in the rat *in vivo* and *in vitro*.

- To study the effect of different concentrations of some vasodilators on reperfusion induced arrhythmias in the *in vitro* model using the constant head of pressure system and in the anaesthetized rat model when given either before ligation or just before reperfusion.

- To test the effects of allopurinol and trypsin inhibitor on reperfusion arrhythmias and superoxide production in the isolated rat heart when administered to animals before study.

- To investigate the effects of d-adrenaline and l-adrenaline on reperfusion arrhythmias and superoxide production in the presence and absence of superoxide dismutase in order to confirm or negate the involvement of auto-oxidation of catecholamines in the arrhythmogenesis during reperfusion.

- To study the effect of traces of Cu^{+} on reperfusion arrhythmias (Cu^{+} was found in preliminary experiments to have a potent cardiotoxic action) and to investigate the expected involvement of free radicals in mediating this effect.

- To investigate the effects of vitamin E deficiency and glutathione depletion on the severity of reperfusion arrhythmias, $^{86}Rb^{+}$ efflux rate constant, ^{3}H-noradrenaline release and lipid peroxidation.

- To study the relationship between atherosclerosis and reper-

fusion induced arrhythmias in the anaesthetized rat model.

- To investigate the effects of reperfusion with anoxic and oxygenated blood in the presence and absence of oxygen free radical scavengers and allopurinol on cardiac arrhythmias, percentage mortility and serum lipid peroxides due to reperfusion after whole body ischaemia (induced by circulatory shock).

REFERENCES

References

Abrahamsson, T. & Almgren, O. (1981). Ventricular fibrillation following coronary artery ligation in the rat. In: Budden, R. *et al.*, eds. The rat electrocardiogram in pharmacology and toxicology. Pergamon Press: Oxford, England, 239-241.

Abrahamsson, T.; Almgren, O. & Carlsson, L. (1983). Ischaemia-induced noradrenaline release in the isolated rat heart: influence of perfusion substrate and duration of ischaemia. J. Mol. Cell. Cardiol. **15**, 821-830.

Abrahamsson , T.; Almgren, O. & Carlsson, L. (1984). Wash-out of noradrenaline and its metabolites by calcium-free reperfusion after ischaemia: support for the concept of ischaemia-induced noradrenaline release. Br. J. Pharmac. **81**, 22-24.

Altura, B.M.; Altura, B.T.; Carella, A. & Turlapaty, P.D.M.V. (1981). Hypomagnesaemia and vasoconstriction: possible relationship to etiology of sudden death ischaemic heart disease and hypertensive vascular diseases. Artery **9**, 212-231.

Altura, B.M. & Altura, B.T. (1982). Mg, Na and K interactions and coronary heart diseases. Magnesium **1**, 241-265.

Andersson, K.E.(1980). The heart cell-electrophysiological aspects. Acta Med. Scand., Suppl. **647**, 7-15.

Axelrod, P.J.; Verrier, R.L. & Lown, B. (1975). Vulnerability of ventricular fibrillation during coronary artery occlusion and release. Am. J. Cardiol. **36**, 776-782.

Balke, C.W.; Kaplinsky, E.; Michelson, E.L.; Naito, M. & Dreifus, L.S. (1981). Reperfusion ventricular tachyarrhythmias: correlation with antecedent coronary artery occlusion tachyarrhythmias and duration of myocardial ischaemia. Am. Heart J. **101**, 449-456.

Band, D.M.: Lim, M.; Linton, R.A.F. & Wolff,C.B.(1982). Changes in arterial plasma potassium during exercise. J. Physiol. **328**, 74P.

Benfey, B.G.; Elfellah, M.S.; Ogilvie, R.I. & Varma, D.R. (1984). Anti-arrhythmic effects of prazosin and propranolol during coronary artery occlusion and reperfusion in dogs and pigs. Br. J. Pharmac. **82**, 717-725.

Bergey, J.L.; Nocella, K. & McCallum, J.D. (1982). Acute coronary artery occlusion - reperfusion - induced arrhythmias in rats, dogs and pigs: antiarrhythmic evaluation of quinidine, procainamide and lidocaine. Eur. J. Pharmac. **81**, 205-216.

Bergey, J.L. & Beil, M.E. (1983). Antiarrhythmic evaluation of naloxone against acute coronary occlusion induced arrhythmias in pigs. Eur. J. Pharmac. **90**, 427-431.

Bergey, J.L.; Wendt, R.L.; Nocella, K. & McCallum, J.D. (1984). Acute coronary artery occlusion - reperfusion arrhythmias in pigs: antiarrhythmic and antifibrillatory evaluation of verapamil, nifedipine, prenylamine and propranolol. Eur. J. Pharmac. **97**, 95-103.

Blackwell, G.J. & Flower, R.J. (1983). Inhibition of phospholipase. Br. Med. Bull. **39**, 260-264.

Bolli, R.; Fisher, D.J.; Taylor, A.A.; Young, J.B. & Miller, R.R. (1984). Effect of α-adrenergic blockade on arrhythmias induced by acute myocardial ischaemia and reperfusion in the dog. J. Mol. Cell. Cardiol. **16**, 1101-1117.

Bolton, T.B. & Clark, J.P. (1981). Effects of histamine, high potassium and carbacol on $^{42}K^{+}$ efflux from longitudinal muscle of guinea-pig intestine. J. Physiol. **320**, 347-361.

Bralet, J.; Didier, J.P.; Moreau, D.; Opie, L.H. & Rochette, L. (1985). Effect of α-adrenoceptor antagonists (phentolamine, nicergoline and prazosin) on reperfusion arrhythmias and noradrenaline release in perfused rat heart. Br. J. Pharmac. **84**, 9-18.

Brawn, K. & Fridovich, I. (1980).Superoxide radical and superoxide dismutases: threat and defense. Acta Physiol. Scand., Suppl. **492**, 9-18.

Bunting, S.; Moncada, S. & Vane, J.R. (1983). The prostacyclin-thromboxane A_2 balance: pathophysiological and therapeutic implications. Br. Med. Bull. **39**, 271-276.

Burton, K.P.; McCord, J.M. & Ghai, G. (1984). Myocardial alterations due to free radical generation. Am. J. Physiol. **246**, H776-H783.

Carbonin, P.; Di Gennaro, M.; Valle, R. & Weisz, A.M. (1981). Inhibitory effect of anoxia on reperfusion and digitalis-induced ventricular tachyarrhythmias. Am. J. Physiol. **240**, H730-H737.

Chambers, D.E.; Parks, D.A.; Patterson, G.; Roy, R.; McCord, J.M.; Yoshida, S.; Parmley, L.F. & Downey, J.M. (1985). Xanthine oxidase as a source of free radical damage in myocardial ischaemia. J. Mol. Cell. Cardiol. **17**, 145-152.

Chien, K.R.; Han, A.; Sen,A.; Buja, L.M. & Willerson, J.T. (1984). Accumulation of unesterified arachidonic acid in ischaemia canine myocardium. Relationship to a phosphatidylcholine deacylation-reacylation cycle and the depletion of membrane phospholipids. Circ. Res. **54**, 313-322.

Clark, C.; Foreman, M.I.; Kane, K.A.; McDonald, F.M. & Parratt, J.R. (1980).Coronary artery ligation in anaesthetized rats as a method for the production of experimental dysrhythmias

and for the determination of infarct size. J. Pharmacol. Methods. **3**, 357-368.

Clusin, W.T.; Bristow, M.R.; Karagueuzian, H.S.; Katzung, B.G. & Schroeder, J.S. (1982). Do calcium-dependent ionic currents mediate ischaemic ventricular fibrillation?. Am. J. Cardiol. **49**, 606-612.

Coker, S.J.; Parratt, J.R., Ledingham, I. McA. & Zeitlin, I.J. (1981). Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. Nature.**291**,323-324.

Coker, S.J. (1982). Early ventricular arrhythmias arising from acute myocardial ischaemia, possible involvement of prostaglandins and thromboxanes. In: Parratt, J.R. ed. Early arrhythmias resulting from myocardial ischaemia. Macmillan Press, London, England, 219-237.

Coker, S.J., Parratt, J.R.; Ledingham, I.McA. & Zeitlin, I.J. (1982). Evidence that thromboxane contributes to ventricular fibrillation induced by reperfusion of the ischaemic myocardium. J. Mol. Cell. Cardiol. **14**, 483-485.

Coker, S.J.& Parratt, J.R. (1983a) Nifedipine reduces arrhythmias but does not alter prostanoid release during coronary artery occlusion and reperfusion in anaesthetized greyhounds. J. Cardiovasc. Pharmac. **5**, 406-417.

Coker, S.J. & Parratt, J.R. (1983b). Prostacyclin-antiarrhythmic or arrhythmogenic? Comparison of the effects of intravenous and intracoronary prostacyclin and ZK 36374 during coronary artery occlusion and reperfusion in anaesthetized greyhounds. J. Cardiovasc. Pharmac. **5**, 557-567.

Coker, S.J. (1984). Further evidence that thromboxane exacerbates arrhythmias: effects of UK 38485 during coronary artery

occlusion and reperfusion in anaesthetized greyhounds. J. Mol. Cell. Cardiol. **16**, 633-641.

Coker, S.J. & Parratt, J.R. (1984a). Antiarrhythmic activity of the thromboxane antagonist AH 23848 during canine myocardial ischaemia and reperfusion. Br. J. Pharmac. **83**, 380P.

Coker, S.J. & Parratt, J.R. (1984b). The effects of nafazatrom on arrhythmias and prostanoid release during coronary artery occlusion and reperfusion in anaesthetized greyhounds. J. Mol. Cell.. Cardiol. **16**, 43-52.

Corbalan, R.; Verrier, R.L. & Lown, B. (1976). Differing mechanisms for ventricular vulnerability during coronary artery occlusion and release. Am. Heart J. **92**, 223-230.

Corr, P.B.;Gross, R.W. & Sobel, B.E. (1982). Arrhythmogenic amphiphilic lipids and the myocardial cell membrane. J. Mol. Cell Cardiol. **14**, 619-626.

Corr, P.B. & Witkowski, F.X. (1983). Potential electrophysiologic mechanisms responsible for dysrhythmias associated with reperfusion of ischaemic myocardium. Circ. **68**, I16-I24.

Corr, P.B. & Witkowski, F.X. (1984). Arrhythmias associated with reperfusion: basic insights and clinical relevance. J. Cardiovasc. Pharmac. **6**, S903-S909.

Corr, P.B.; Gross, R.W. & Sobel, B.E. (1984). Amphipathic metabolites and membrane dysfunction in ischaemic myocardium. Circ. Res. **55** , 135-154.

Cranfield, P.E. & Wit, A.L. (1979). Cardiac arrhythmias. Ann. Rev. Physiol. **41**, 459-472.

Crome, R.; Hearse, D. & Manning,A. (1983). Relationship between cellular cyclic AMP content and the incidence of ventricular

fibrillation upon reperfusion after varying periods of ischaemia. J. Mol. Cell. Cardiol. **15**, 180 (abstract).

Crook, D.; Collins, A.J.; Bacon, P.A. & Chan, R. (1976). Prostaglandin synthetase activity from human rheumatoid synovial microsomes. Ann.Rheum. Dis. **35**, 327-332.

Culling, W.; Penny, W.J.; Lewis, M.J.; Middleton, K. & Sheridan, D.J. (1984). Effects of myocardial catecholamine depletion on cellular electrophysiology and arrhythmias during ischaemia and reperfusion. Cardiovasc. Res. **18**, 675-682.

De Deckere, E.A.M.; Nugteren, D.H. & Hoor,F.T. (1977). Prostacyclin is the major prostaglandin released from the isolated perfused rabbit and rat heart. Nature.**268**, 160-163.

De Mello, W.C. (1982). Intercellular communication in cardiac muscle. Circ. Res. **51**, 1-9.

De Wall, R.A.; Vasko, K.A.; Stanley, E.L. & Kezdi, P. (1971). Responses of the ischaemic myocardium to allopurinol. Am. Heart J. **82**, 362-370.

Dietz, R. (1983). The role of potassium in hypertension. Am. J. Nephrol. **3**, 100-108.

Dormandy, T.L.(1978). Free-radical oxidation and antioxidants. Lancet. **1**, 647-650.

Downar, E.; Janse, M.J. & Durrer, D. (1977a). The effect of ischaemic blood on transmembrane potentials of normal porcine ventricular myocardium. Circ. **55**, 455-462.

Downar, E.; Janse, M.J. & Durrer, D. (1977b). The effect of acute coronary artery occlusion on subepicardial transmembrane potentials in the intact porcine heart. Circ. **56**, 217-224.

Duniec, Z.; Robak, J. & Gryglewski, R. (1983). Antioxidant properties of some chemicals vs. their influence on

cyclooxygenase and lipoxygenase activities. Biochem. Pharmac. **32**, 2283-2286.

Durbin, R.P. & Jenkinson, D.H. (1961). The effect of carbachol on the permeability of depolarized smooth muscle to inorganic ions. J Physiol. **157**, 74-89.

Dyckner, T. & Wester, P.O. (1980). Relation between potassium, magnesium and cardiac arrhythmias. Acta Med. Scand., Suppl. **647**, 163-169.

Falanga, A.; Doni,M.G.; Delaini, F.; Vitti, G.D.B.; Imberti, L.; Donati, M.B. & Gaetano, G.D. (1983). Unbalanced plasma control of TXA_2 and PGI_2 synthesis in vit. E. deficient rats. Am. J. Physiol. **245**, H867-H870.

Fisch, C. (1973). Relation of electrolyte disturbances to cardiac arrhythmias. Circ. **47**, 408-419.

Frank. L. (1983). Superoxide dismutase and lung toxicity . TIPS. March, 124-128.

Gettes, L.S. (1981). Possible role of ionic changes in the appearance of arrhythmias. In: Szeker, L. ed. Pharmacology of antiarrhythmic drugs. Pergamon Press: Oxford, England. 41-64.

Goldberg, S.; Greenspon, A.J.; Urban, P.L. ; Muza, B.; Berger, B.; Walinsky, P. & Maroko, P.R. (1983). Reperfusion arrhythmia: a marker of restoration of antegrade flow during intracoronary thrombolysis for acute myocardial infarction. Am. Heart J. **105**, 26-32.

Goldstein, I.m.: Roos, D.; Kaplan, H.B. & Weissmann, G. (1975). Complement and immunoglobulins stimulate superoxide production by human leukocytes independently of phagocytosis. J.Clin. Invest. **56**, 1155-1163.

Gutteridge, J.M.C. (1982). The role of superoxide and hydroxyl radicals in phospholipid peroxidation catalysed by iron salts. FEBS Letters. **150**, 454-458.

Halliwell, B. (1981). Free radicals, oxygen toxicity and aging. In: Sohab, R.S., ed., Age Pigments. Elsevier Biomedical Press: Amsterdam, Holland, 1-26.

Halliwell, B. & Gutteridge, J.M.C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. **219**, 1-14.

Harris, A.S., Bisteni, A., Russell, R.A., Brigham, J.C. & Firestone, J.E. (1954). Excitatory factors in ventricular tachycardia resulting from myocardium ischaemia. Potassium a major excitant. Science **119**, 200-203.

Henquin, J.C. (1977). Tetraethylammonium potentiation of insulin release and inhibition of rubidium influx in pancreatic islets. Biochem. Biophys. Res. Commun. **77**, 551-556.

Hertz, F. & Cloarec, A. (1984). Pharmacology of free radicals, recent views on their relation to inflammatory mechanisms. Life Sciences **34**, 713-720.

Hess, M.L., Okabe, E. & Kontos, H.A. (1981). Proton and free oxygen radical interaction with the calcium transport system of cardiac sarcoplasmic reticulum. J. Mol. Cell. Cardiol.**13**, 767-772.

Hess, M.L. & Manson, N.H. (1984). Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischaemia/reperfusion injury. J. Mol. Cell. Cardiol. **16**, 969-985.

Higgs, G.A. & Vane, J.R. (1983). Inhibition of cyclo-oxygenase and lipoxygenase. Br. Med. Bull. **39**, 265-270.

Hohorst, H.J. (1965). L-(+) Lactate determination with lactic

dehydrogenase and DPN. In: Bergmeyer, H.U. ed., Methods in enzymatic analysis. Academic Press, New York, U.S.A., 266-270.

Imaizumi, Y. & Watanabe, M. (1981). The effect of tetraethylammonium chloride on potassium permeability in the smooth muscle cell membrane of canine trachea. J. Physiol. **316**, 33-46.

Kane, K.A., Parratt, J.R. & Williams, F.M. (1984). An investigation into the characteristics of reperfusion-induced arrhythmias in the anaesthetized rat and their susceptibility to antiarrhythmic agents. Br. J. Pharmac. **82**, 349-357.

Kaplinsky, E., Ogawa, S., Michelson, E.L. & Dreifus, L.S. (1981). Instantaneous and delayed ventricular arrhythmias after reperfusion of acutely ischaemic myocardium: evidence for multiple mechanisms. Circ. **63**, 333-340.

Kloner, R.A., Ellis, S.G., Lange, R. & Braunwald, E. (1983). Studies of experimental coronary artery reperfusion. Effects on infarct size, myocardial function, biochemistry, ultra-structure and microvascular damage. Circ. **68**, I8-I15.

Kontos, H.A., Wei, E.P., Povlishock, J.T. & Christman, C.W. (1984). Oxygen radicals mediate the cerebral arteriolar dilation from arachidonate and bradykinin in cats. Circ. Res. **55**, 295-303.

Koster, J.F., Slee, R.G., Essed, C.E. & Stam, H. (1985). Studies on cumene hydroperoxide-induced lipid peroxidation in the isolated rat heart. J. Mol. Cell. Cardiol. **17**, 701-708.

Iseri, L.T., Freed, J. & Bures, A.R. (1975). Magnesium deficiency and cardiac disorders. Am. J. Med. **58**, 837-846.

Janse, M. (1982). Electrophysiological changes in the acute phase of myocardial ischaemia and mechanisms of ventricular arrhythmias. In: Parratt, J.R., ed. Early arrhythmias resulting from

myocardial ischaemia. Macmillan Press, London, England, 57-80.

Johnson, C.J., Peterson, D.R. & Smith, E.K. (1979). Myocardial tissue concentrations of Mg^{2+} and K^{+} in men dying suddenly from ischaemic heart disease. Am. J. Clin. Nutr. **32**, 967-970.

Johnston, K.M., MacLeod, B.A. & Walker, M.J.A. (1983). Effects of aspirin and prostacyclin on arrhythmias resulting from coronary artery ligation and on infarct size. Br. J. Pharmac. **78**, 29-37.

Jolly, S.R., Kane, W.J., Bailie, M.B., Abrams, G.D. and Lucchesi, B.R. (1984). Canine myocardial reperfusion injury. its reduction by the combined administration of superoxide dismutase and catalase. Circ. Res. **54**, 277-285.

Kuehl, F.A., Dougherty, H.W. & Ham, E.A. (1984). Interactions between prostaglandins and leukotrienes. Biochem.Pharmac. **33**, 1-5.

Levites, R., Banka, V.S. & Helfant, R.H. (1975). Electrophysiologic effects of coronary occlusion and reperfusion. Observations of dispersion of refractoriness and ventricular automaticity. Circ. **52**, 760-765.

Linton, R.A.F., Lim, M. and Band, D.M. (1982). Continuous intravascular monitoring of plasma potassium using potassium-selective electrode catheters. Crit. Care Med. **10**, 337-340.

Lubbe, W.F., Daries, P.S. & Opie, L.H. (1978). Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of antifibrillatory action of antiarrhythmic agents. Cardiovasc. Res. **12**, 212-220.

Lynch, R.E. & Fridovich, I. (1978). Effects of superoxide on the erythrocyte membrane. J. Biol. Chem. **253**, 1838-1845.

Magovern, G.J., Bolling, S.F., Casale, A.S., Bulkley, B.H. & Gardner, T.J. (1984). The mechanism of mannitol in reducing ischaemic injury: hyperosmolarity or hydroxyl scavenger? Circ. **70**, I91-I95.

Manning, A.S. & Hearse, D.J. (1984). Reperfusion induced arrhythmias: mechanisms and prevention. J. Mol. Cell. Cardiol. **16**, 497-518.

Matthews, E.K. & Shotton, P.A. (1984). Efflux of $^{86}Rb^{+}$ from rat and mouse pancreatic islets: the role of membrane depolarization. Br. J. Pharmac. **83**, 831-839.

McCord, J.M. (1985). Oxygen-derived free radicals in postischaemic tissue injury. The new Eng. J. Med. **312**, 159-163.

Meerson, F.Z., Kagan, V.E., Kozlov, Yu.P., Belkina, L.M. & Arkhipenko, Yu. V. (1982). The role of lipid peroxidation in pathogenesis of ischaemic damage and the antioxidant protection of the heart. Basic Res. Cardiol. **77**, 465-485.

Mimnaugh, E.G., Gram, T.E. & Trush, M.A. (1983). Stimulation of mouse heart and liver microsomal lipid peroxidation by anthracycline anticancer drugs: characterization and effects of reactive oxygen scavengers. J. Pharmac. Exp. Ther. **226**, 806-815.

Moschos, C.B., Haider, B., De La Cruz, C., Lyons, M.M. & Regan, T.J. (1978). Antiarrhythmic effects of aspirin during nonthrombotic coronary occlusion. Circ. **57**, 681-684.

Mullane, K.M. & Moncada, S. (1982). The salvage of ischaemic myocardium by BW 755C in anaesthetised dogs. Prostaglandins. **24**, 255-266.

Mullane, K.M., Read, N., Salmon, J.A. & Moncada, S. (1984). Role of leucocytes in acute myocardial infarction in anaesethetised dogs: relationship to myocardial salvage by anti-inflammatory

drugs. J. Pharmac. Exp. Ther. **228**, 510-521.

Murdock, D.K., Loeb, J.M., Euler, D.E. & Randall, W.C. (1980). Electrophysiology of coronary reperfusion. A mechanism for reperfusion arrhythmias. Circ. **61**, 175-182.

Naimi, S., Avitall, B., Meiszala, J. & Levine, H.J. (1977). Dispersion of effective refractory period during abrupt reperfusion of ischaemic myocardium in dogs. Am. J. Cardiol. **39**, 407-412.

Nayler, W.G. (1980). Cardioprotective effects of calcium ion antagonists in myocardial ischaemia. Clin. Invest. Med. **3**, 91-99.

Nayler, W.G. (1981a). The heart cell: some metabolic aspects of cardiac arrhythmias. Acta. Med. Scand. Suppl. **647**, 17-31.

Nayler, W.G. (1981b). The role of calcium in the ischaemic myocardium. Am. J. Pathol. **102**, 262-270.

Nayler, W.G. & Sturrock, W.J. (1984). An inhibitory effect of verapamil and diltiazem on the release of noradrenaline from ischaemic and reperfused hearts. J. Mol. Cell. Cardiol. **16**, 331-344.

Nelson, D.J. & Elion, G.B. (1975). Mechanism of 6-^{14}C allopurinol - lack of incorporation of allopurinol into nucleic acids. Biochem. Pharmac. **24**, 1235-1237.

Nishikimi, M. (1975). Oxidation of ascorbic acid with superoxide anion generated by the xanthine-xanthine oxidase system. Biochem. Biophysic. Res. Commun. **63**, 463-468.

Opie, L.H., Nathan, D. & Lubbe, W.F. (1979). Biochemical aspects of arrhythmogenesis and ventricular fibrillation. Am. J. Cardiol. **43**, 131-148.

Otani, H., Tanaka, H., Inoue, T., Umemoto, M., Omoto, K., Tanaka, K., Sato, T., Osako, T., Masuda, A., Nonoyama, A. & Kagawa, T.

(1984). *In vitro* study on contribution of oxidative metabolism of isolated rabbit heart mitochondria to myocardial reperfusion injury. Circ. Res. **55**, 168-175.

Parmley, W.W. & Chatterjee, K. (1984). New concepts in ischaemic heart disease. J. Cardiovasc. Pharmac. **6**, 888S - 894S.

Parratt, J.R. (1982). Calcium slow channel blocking antagonists and early ventricular arrhythmias resulting from myocardial ischaemia and subsequent reperfusion. In: Godfraind, T. *et al.*, eds. Elsevier Biomedical Press; Amsterdam, Holland, 87-98.

Patrignani, P., Filabozzi, P., Catella, F., Pugliese, F. & Patrono, C. (1984). Differential effects of dazoxiben,a selective thromboxane synthase inhibitor on platelet and renal prostaglandin endoperoxide metabolism. J. Pharmac. Exp. Ther. **228**, 472-477.

Petropoulos, P.C. & Meijne, N.G. (1964). Cardiac function during perfusion of the circumflex coronary artery with venous blood, low molecular dextran or tyrode solution. Am. Heart J. **68**, 370-382.

Piper, P.J. (1983). Pharmacology of leukotriens. Br. Med. Bull. **39**, 255-259.

Ramanathan, K.B., Bodenheimer, M.M., Banka, V.S. & Helfant,R.H. (1977). Electrophysiologic effects of partial coronary occlusion and reperfusion. Am. J. Cardiol. **40**, 50-54.

Rao, P.S., Cohen, M.V. & Mueller, H.S. (1983). Production of free radicals and lipid peroxides in early experimental myocardial ischaemia. J. Mol. Cell. Cardiol. **15**, 713-716.

Reimer, K.A. & Jennings, R.B. (1985). Failure of the xanthine

oxidase inhibitor allopurinol to limit infarct size after ischaemia and reperfusion in dogs. Circ. **71**, 1069-1075.

Riemersma, R.A. (1982). Myocardial catecholamine release in acute myocardial ischaemia: relationship to cardiac arrhythmias. In: Parratt, J.R., ed. Early arrhythmias resulting from myocardial ischaemia. Macmillan Press, London, England, 125-138.

Rochette, L., Didier, J.P., Moreau, D. & Bralet, J. (1980). Effect of substrate on release of myocardial norepinephrine and ventricular arrhythmias following reperfusion of the ischaemic isolated working rat heart. J. Cardiovasc. Pharmac. **2**, 267-279.

Rowe, G.T., Manson, N.H., Caplan, M. & Hess, M.L. (1983). Hydrogen peroxide and hydroxyl radical mediation of activated leukocyte depression of cardiac sarcoplasmic reticulum. Participation of the cyclo-oxygenase pathway. Circ. Res. **53**, 584-591.

Saman, S. & Opie, L.H. (1984). Mechanism of reduction of action potential duration of ventricular myocardium by exogenous lactate. J. Mol. Cell. Cardiol. **16**, 659-662.

Schömig, A., Dart, A.M., Dietz, R., Mayer, E. & Kübler, W. (1984). Release of endogenous catecholamines in the ischaemic myocardium of the rat. Part A: Locally mediated release. Circ. Res. **55**, 689-701.

Schoutsen, B., De Jong, J.W., Harmsen, E., De Tombe, P.P. & Achterberg, P.W. (1983). Myocardial xanthine oxidase/ dehydrogenase. Biochimica et Biophysica Acta **762**, 519-524.

Schwartz, A.B. (1978). Potassium-related cardiac arrhythmias and their treatment. Angiology **29**, 194-205.

Seelig, M.S. & Heggtveit, H.A. (1974). Magnesium interrelationships in ischaemic heart disease:a review. Am. J. Clin. Nutrit. **27**, 59-79.

Sewell, W.H., Koth, D.R.& Huggins, C.(1955). Ventricular fibrillatio n in dogs after sudden return of flow to the coronary artery. Surgery **38**, 1050-1053.

Sheridan, D.J., Penkoske, P.A., Sobel, B.E. & Corr, P.B. (1980). Alpha adrenergic contributions to dysrhythmias during myocardial ischaemia and reperfusion in cats. J. Clin. Invest. **65**, 161-171.

Shine, K.I. & Douglas, A.M. (1974). Magnesium effects on ionic exchange and mechanical function in rat ventricle. Am. J. Physiol. **227**, 317-324.

Shine, K.I. & Douglas, A.M. (1975). Magnesium effect on rabbit ventricle. Am. J. Physiol. **228**, 1545-1554.

Shine, K.I., Douglas, A.M. & Ricchiuti, N.V. (1978). Calcium, strontium and barium movements during ischaemia and reperfusion in rabbit ventricle. Circ. Res. **43**, 712-720.

Shine, K.I. (1979). Myocardial effects of magnesium. Am. J. Physiol. **237**, H413-H423.

Shine, K.I. & Douglas, A.M. (1983). Low calcium reperfusion of ischaemic myocardium. J. Mol. Cell. Cardiol. **15**, 251-260.

Simchowitz, L. & Spilberg, I. (1979). Generation of superoxide radicals by human peripheral neutrophils activated by chemotactic factor. Evidence for the role of calcium. J. Lab. Clin. Med. **93**, 583-593.

Singal, P.K., Beamish, R.E. & Dhalla, N.S. (1983). Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. Adv. Exp. Med. Biol. **161**, 391-401.

Sobel, B.E., Corr, P.B., Robison, A.K., Goldstein, R.A., Witkowski, F.X. & Klein, M.S. (1978). Accumulation of lysophosphoglycerides with arrhythmogenic properties in ischaemic myocardium. J. Clin. Invest. **62**, 546-553.

Spear, J.F. (1981). Relationship between the scaler electrocardiogram and cellular electrophysiology of the rat heart. In: Budden R. *et al.*, eds. The rat electrocardiogram in pharmacology and toxicology. Pergamon Press: Oxford, England, 29-40.

Swain, J.L., Sabina, R.L., Hines, J.J., Greenfield Jr., J.C. & Holmes, E.W. (1984). Repetitive episodes of brief ischaemia (12 min) do not produce a cumulative depletion of high energy phosphate compounds. Cardiovasc. Res. **18**, 264-269.

Taylor, G.W. & Morris, H.R. (1983). Lipoxygenase pathways. Br. Med. Bull. **39**, 219-222.

Thandroyen, F.T., Worthington, M.G., Higginson, L.M. & Opie, L.H. (1983). The effect of alpha and beta-adrenoceptor antagonist agents on reperfusion ventricular fibrillation and metabolic status in the isolated perfused rat heart. J.Am. Coll. Cardiol. **1**, 1056-1066.

Tomita, T. & Yamamoto, T. (1971). Effects of removing the external potassium on the smooth muscle of guinea-pig taenia coli. J. Physiol. **212**, 851-868.

Trush, M.A., Mimnaugh, E.G. & Gram, T.E. (1982). Activation of pharmacologic agents to radical intermediates. Implications for the role of free radicals in drug action and toxicity. Biochem. Pharmac. **31**, 3335-3346.

Van der Vusse, G.J. & Reneman, R.S. (1985). Pharmacological intervention in acute myocardial ischaemia and reperfusion. TIPS. Feb, 76-79.

Vassalle, M. (1981). Generation and conduction of impulses in the heart under physiological and pathological conditions. In: Szekeres, L., ed. Pharmacology of antiarrhythmic drugs. Pergamon Press: Oxford, England, 1-40.

Ward, J.P.T. & Cameron, I.R. (1985). Alterations to potassium fluxes in the heart during chronic dietary potassium depletion. J. Mol. Cell. Cardiol. **17**, 597-602.

Weksler, B.B. (1984).Prostaglandins and vascular function. Circ. **70**, III63-III71.

Whang, R., Oei, T.O., Aikawa, J.K., Ryan, M.P., Watanabe, A., Chrysant, S.G. & Fryer, A. (1980). Magnesium and potassium interrelationships experimental and clinical. Acta. Med. Scand. Suppl. **647**, 139-144.

Wheatley, A.M., Thandroyen, F.T. & Opie, L.H. (1985). Catecholamine-induced myocardial cell damage: catecholamines or adrenochrome. J. Mol. Cell. Cardiol. **17**, 349-359.

Whittle, B.J.R. & Moncada, S. (1983). Pharmacological interactions between prostacyclin and thromboxanes. Br. Med. Bull. **39**, 232-238.

Winslow, E., Marshall, R.J. & Hope, F.G. (1983). Comparative effects of fast and slow-ion channel blocking agents on reperfusion-induced arrhythmias in the isolated perfused rat heart. J. Cardiovasc. Pharmac. **5**, 928-936.

Woodward, B. (1981). A model of ventricular fibrillation in the isolated rat heart. J. Pharmacol. Methods **6**, 219-231.

Woodward, B. & Zakaria, M.N.M. (1983). Effects of potassium and magnesium on reperfusion induced arrhythmias in the isolated rat heart following coronary artery ligation. J. Physiol. **343**, 55-56P.

Woodward, B. & Zakaria, M.N.M. (1985). Effects of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart. J. Mol. Cell. Cardiol. **17**, 485-493.

List of Publications

1. Woodward B. and Zakaria M.N.M. The effects of potassium and magnesium on reperfusion induced arrhythmias in the isolated rat heart following coronary artery ligation. J. Physiol. **343**, 55-56P, 1983.
2. Woodward, B., Hassan, M.N.M.Z. and Mohamed, O.Y. Effects of potassium on ischaemic and reperfusion arrhythmias in the isolated rat heart. J. Mol. Cell. Cardiol. **15**, 414C.
3. Woodward, B. and Zakaria, M.N.M. The effects of free radical scavengers on reperfusion arrhythmias in the isolated rat heart. Br. J. Pharmac. **80**, 628P, 1983.
4. Woodward,B. and Zakaria, M.N.M. Effect of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart. J. Mol. Cell. Cardiol. **17**, 485-493, 1985.
5. Woodward, B. and Zakaria, M.N.M. Evidence that free radicals may contribute to the development of reperfusion induced arrhythmias in the isolated rat heart. J. Mol. Cell. Cardiol. **16**, 8C, 1984.
6. Avkiran, M., Daugherty, A., Woodward, B. & Zakaria, M.N.M. Antiarrhythmic actions of Desmethylimipramine in the rat. J. Mol. Cell. Cardiol. **16**, 57C, 1984.